JOURNAL OF AGRICULTURAL RESEARCH

VOL. 49 WASHINGTON, D. C., DECEMBER 1, 1934 No. 11

RELATION OF LEAF-RUST INFECTION TO YIELD, GROWTH, AND WATER ECONOMY OF TWO VARIETIES OF WHEAT [1]

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INTRODUCTION

During the course of investigations at the Kansas Agricultural Experiment Station on the nature of leaf rust (*Puccinia triticina* Eriks.) and its effect on the wheat plant, it has become increasingly evident that the parasite has a marked effect on the physiology of the host. Some observations have indicated a serious effect on the yield and on certain plant characters, and it also has frequently been noted that heavily rusted wheat plants in the greenhouse suffered more severely from lack of water than did rust-free plants. The experiments herein described were planned to obtain further light on the nature and causes of reductions in yield because of leaf-rust infection, to determine the effect of such infection on growth and plant characters, and to establish the relation of leaf rust to the economy of the wheat plant in its use of water. It was also planned to study the interrelation of these various factors and to establish a basis for researches on the relation of leaf-rust infection to the physiology of the host plant.

HISTORICAL REVIEW

The literature on cereal rusts and their effect on the host plants is extensive, but the researches discussed in this paper do not demand mention of much of it. References to the effects of rust on yield of wheat deal mostly with stem rust (*Puccinia graminis* Pers.) and relatively little with leaf rust. Until rather lately there has been no general agreement as to the effect of the latter. Thus, Carleton (*3*),[3] Eriksson and Henning (*5*), and McAlpine (*12*) considered leaf rust a disease of rather minor importance, causing very little damage, whereas Cobb (*4*), Klebahn (*11*), and Grove (*6*) are emphatic in their statements that heavy infections of leaf rust caused pronounced losses. Hayes, Aamodt, and Stevenson (*7*) found that leaf-rust infection had a definitely depressing effect on the yield of some varieties of spring wheat. Kightlinger and Whetzel (*10*) studied the control of cereal rusts by dusting with sulphur and found that winter wheat plots dusted with sulphur yielded 18.5 percent more than the heavily rusted

[1] Received for publication July 17, 1934; issued February 1935. Contribution no. 342 from the Department of Botany, Kansas Agricultural Experiment Station. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station.

[2] The writers gratefully acknowledge indebtedness to H. B. Humphrey, of the Division of Cereal Crops and Diseases, for valuable suggestions and criticisms during the course of the experiments.

[3] Reference is made by number (italic) to Literature Cited, p. 980.

controls. Tehon (*17*), measuring destructiveness in terms of prevalence and severity of rust, leaves the impression that leaf rust was the cause of considerable loss in the yield of wheat in Illinois from 1922 to 1926, inclusive. More recently Mains (*13*) has definitely proved that leaf rust causes severe losses, especially when it develops in the fall or very early spring. He has further shown that the yield reduction was due principally to a decrease in the number of kernels per head and, in a lesser degree, to a reduction of kernel size. Johnston (*8*) obtained similar results and recorded maximum yield depreciations of 55.7 percent for a susceptible variety and 22.3 percent for a resistant one. Both Mains and Johnston pointed out that the heavy flecking caused by leaf rust on leaves of a resistant variety of wheat was definitely correlated with reduction in yield. Neill (*15*), studying the effects of rusts on yield of wheat in New Zealand, found that combined heavy infections of leaf and stem rusts reduced yields from 17 to 96 percent, depending on the degree of varietal susceptibility. He concluded that the reduction was due chiefly to a diminished weight of individual grains from rusted plants and, to a much smaller extent, to the production of fewer kernels per head. Caldwell et al. (*2*) found that in the field leaf rust sometimes reduced yield of grain more than 25 percent. They estimate that about 75 percent of the reduction is traceable to fewer kernels per head and 25 percent to decreased weight per kernel. They also point out that severe leaf-rust infections were accompanied by a lower protein and sucrose content of the grain and a higher starch content. Although they obtained somewhat lower test weights per bushel on grain from rusted plots than from control plots, they observed no shriveling.

Very little of a definite nature concerning the effect of leaf-rust infection on growth characters of the wheat plant can be found in the literature. Mention occasionally is made of reduced plant height and small head size in a rusted crop, but the results of definite experiments designed to measure such factors have very seldom been stated. Mains (*13*), however, noted that heavily rusted plants had smaller heads, a smaller leaf area, and less cold resistance than rust-free control plants. He also observed that, in maturing, the straw of rusted plants of certain varieties failed to color properly.

The effect of rust infection on the use of water by host plants has been reported by only a few investigators. Blodgett (*1*) found that a heavy infection of *Gymnoconia interstitialis* (Schl.) Lagh. nearly doubled the loss of water in a species of *Rubus*. Reed and Cooley (*16*), after studies on the effect of *Gymnosporangium juniperi-virginianae* Schw. infection on the loss of water by apple leaves, concluded that the unit transpiration was less in infected than in healthy leaves. Weaver (*18*) reported that heavy rust infections on cereals greatly accelerated the rate of transpiration. Weiss (*19*) published results of experiments in 1924, showing that rusted plants had a higher water requirement than nonrusted controls and that increases were of a higher order when based on grain weight than when based on total dry matter. He states also that the results were significant only in the case of stem-rust infection. Johnston and Miller (*9*), in a preliminary report on the experiments discussed herein, indicated that heavy leaf-rust infection, occurring early in plant growth, nearly doubled the water requirement of a susceptible variety of wheat.

PRELIMINARY EXPERIMENTS WITH WINTER WHEATS

In planning these experiments it was evident that it would be necessary to control as many of the environmental conditions as possible. This, coupled with the necessity of producing and controlling leaf-rust infection, made it obvious that the experiments must be conducted in the greenhouse. No information, however, was available on the best variety of wheat, on the most desirable size and type of containers, or on the most satisfactory way of controlling and recording water losses or of producing and controlling rust infections.

Preliminary experiments, therefore, were conducted in the greenhouse during the fall and winter of 1929–30 to obtain some information as to the best method of conducting the contemplated investigation. Two varieties of soft red winter wheat were grown in large galvanized-iron cans, each holding about 300 pounds of soil. These cans, which had previously been successfully used by the junior writer in studies on the water requirement of corn, sorghums, and other crops (*14*), were provided with tops that could be sealed in place, leaving only a 4-inch central opening through which the plants were free to grow. Thirty cans were sown with Kawvale (C. I.[4] 8180), a leaf-rust-resistant variety, and an equal number with Fulcaster (Kans. no. 96), a susceptible variety. Five plants were grown to maturity in each container. The cans were mounted on small trucks and weighed at intervals on platform scales, and the water loss was recorded and replaced.

When the plants were fully headed a large cloth cage was built around half of the cans of each variety and the plants were heavily dusted with urediospores of leaf rust, physiologic form 9. The plants were heavily atomized with water and the cloth cage was kept moist by frequent sprayings with tap water. Although the experiment was carefully conducted, this method of inoculation proved unsatisfactory. Only a moderate infection was obtained and the end results were disappointing. The data showed an increase in the water requirement of infected plants of 6.9 percent in the case of the susceptible Fulcaster and 5 percent in the case of the resistant Kawvale. Although the increases in the water requirement of the infected plants over that of the uninoculated controls were small and possibly within the experimental error, they seemed to indicate two things, viz, (1) that even moderate infections of leaf rust increase the water requirement of the wheat plant, and (2) that even in a resistant variety, such as Kawvale, leaf-rust infection causing copious flecking of the leaves increases the water requirement.

A great many difficulties developed during the course of these experiments and it was felt that some changes in methods were necessary. The size and weight of the containers made the recording of water losses an extremely laborious and time-consuming process. They also prohibited the use of ordinary moist chambers following inoculation and forced the construction of cloth enclosures. The large soil mass and abundant water supply induced a vigorous, succulent vegetative growth of the plants. This excessive vegetative growth was accompanied by late heading and, as a result of high seasonal greenhouse temperatures, there were many sterile heads. As a consequence of the heavy growth, the plants were inclined to lodge and infection could not be produced on all leaves. Further-

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

more, the dense mass of leaves and tillers made conditions ideal for mildew infection, which proved to be very difficult to control. For these reasons, the plants in the experiments of 1930–31 were grown in 1-gallon stone jars. The methods used in preparing these cultures and in the inoculation of the plants are described under Methods and Materials. Turkey (C. I. 1558), a highly susceptible hard red winter variety, was substituted for Fulcaster because of the variability of the latter with respect to resistance to leaf rust. It also seemed desirable to learn something of the effect of leaf rust following infection at different stages of plant growth. The plants intended for inoculation were, therefore, divided into three groups and rust infections were initiated in the jointing, booting, and flowering stages, respectively.

Although the stone jars were much easier to handle than the large containers, the results of the experiments of 1930–31 were inconclusive and unsatisfactory. This apparently was largely due to the use of winter wheat. Even in the smaller containers winter varieties, when supplied with an abundance of water, made too great a vegetative growth to allow complete inoculation of all leaves. Furthermore, the plants again failed to head until so late in the spring that they were injured by high greenhouse temperature. The most positive increases in water requirement were those calculated on the dry weights of grain produced by the Turkey group, initially infected in the jointing and booting stages. These increases were 91.4 and 17.1 percent, respectively. Based on total dry matter, the increase in water requirement produced by leaf-rust infection in each of these groups was only 1.1 and 0.77 percent, respectively. The results obtained with the resistant Kawvale were largely negative. In general, the differences obtained were too small or too variable to be considered significant.

EXPERIMENTS WITH SPRING WHEATS

The unsatisfactory results with winter wheat indicated the necessity of using varieties with shorter growing periods, stronger straw, and less tendency to make excessive vegetative growth. It was therefore decided to use spring varieties.

METHODS AND MATERIALS

After a survey of the available spring wheat varieties, two were selected that gave satisfactory results. These were Pusa No. 4 (C. I. 8899) and Warden (C. I. 4994). Pusa No. 4 is an awnless hard white spring variety, highly susceptible to leaf rust, physiologic form 9. It is very early, has stiff straw, and tillers very little. Its extreme earliness and tendency toward a scant leafiness limit its grain-producing capacity. Its high rust susceptibility, strong straw, restricted leaf area, and earliness make it an ideal variety for studies of this nature.

When these studies were initiated, it was impossible to find a common white wheat highly resistant to leaf rust and with the same plant characters as those of Pusa No. 4. Warden, a soft red spring variety, finally was selected because of its strong straw and marked resistance to physiologic form 9. It requires a much longer growing season than Pusa No. 4 and, accordingly, tillers more and is somewhat more leafy. For these reasons it also is a more productive variety. Both varieties, however, matured in time to escape injury from high greenhouse temperature, proved to be very easily handled, and appeared to give highly satisfactory results.

Glazed 1-gallon stone jars were selected as containers. Jars of this size hold sufficient soil to grow six normal wheat plants to maturity and are small enough to facilitate weighing and inoculating operations.

A good quality of dark, fine, sandy loam soil was used. This was sifted to remove lumps and trash, after which it was thoroughly mixed with sifted sand and well-rotted stable manure in the proportion of 6 parts soil, 1 part sand, and 1 part manure. The moisture content of this mixture was then determined and enough water was added to bring the moisture up to about 25 percent, calculated on the basis of weight of dry soil. The actual percentages were 25.3 in 1931–32 and 27.6 in 1932–33.

The jars were filled to the same level, the soil being lightly tamped into them to render it firm but not packed. A core of soil about 2½ inches in diameter and extending about three-fourths of the distance to the bottom was then removed from the center of each jar. At points about 2 inches above the bottom of this excavation three smaller cores extending outward and downward at equally spaced intervals were removed with a ⅝-inch cork borer. These three openings were filled with coarse sand, after which the larger cavity was filled to within about 1 inch of the soil line with coarse gravel. A 2-inch paraffined flowerpot, with greatly enlarged drainage opening, was then pressed firmly into the top of the coarse-gravel core and soil tamped in the space around it to hold it in place. This small pot made a very satisfactory opening through which water could be added to the jar. Loss of water through it was prevented by inverting half of a small Petri dish over the flowerpot. This was easily removed during weighing and watering operations (fig. 1).

In order to prevent the loss of water from the surface of the jars, hot paraffin (in 1931–32) or hot beeswax mixed with venetian turpentine (in 1932–33) was spread in a layer about one-eighth of an inch thick over the soil. These seals adhered readily to the sides of the jar and the small central pot upon hardening but, because of expansion and contraction due to temperature changes, pulled slightly away from the sides of the jar late in the season. After the plants in all jars had emerged, a layer of leached sand was added in the proper quantity to bring each of the jars to the same weight. This also effectually served the purpose of sealing small cracks where the seals had pulled away from the sides of the jars.

In sowing the seed in the jars six equally spaced holes about one-half inch in diameter were cut through the wax layer and one large, well-formed seed was placed in each opening. These seeds were then pressed into the soil about one-half inch with a small blunt instrument and a little water was poured into each opening to insure rapid germination. Thirty-five jars were planted in this manner with each of the two varieties of wheat. This allowed the selection of 5 groups of 6 jars each for each variety for experimentation and gave 5 extra jars of each for use in case of accident or variations among the jars. In addition to these a group of 5 jars in 1931–32 and 2 jars in 1932–33 were not planted but were kept as blank checks on the loss of water because of the set-up.

As soon as the plants had emerged, the jars were weighed and this original weight was recorded. Thereafter they were weighed and

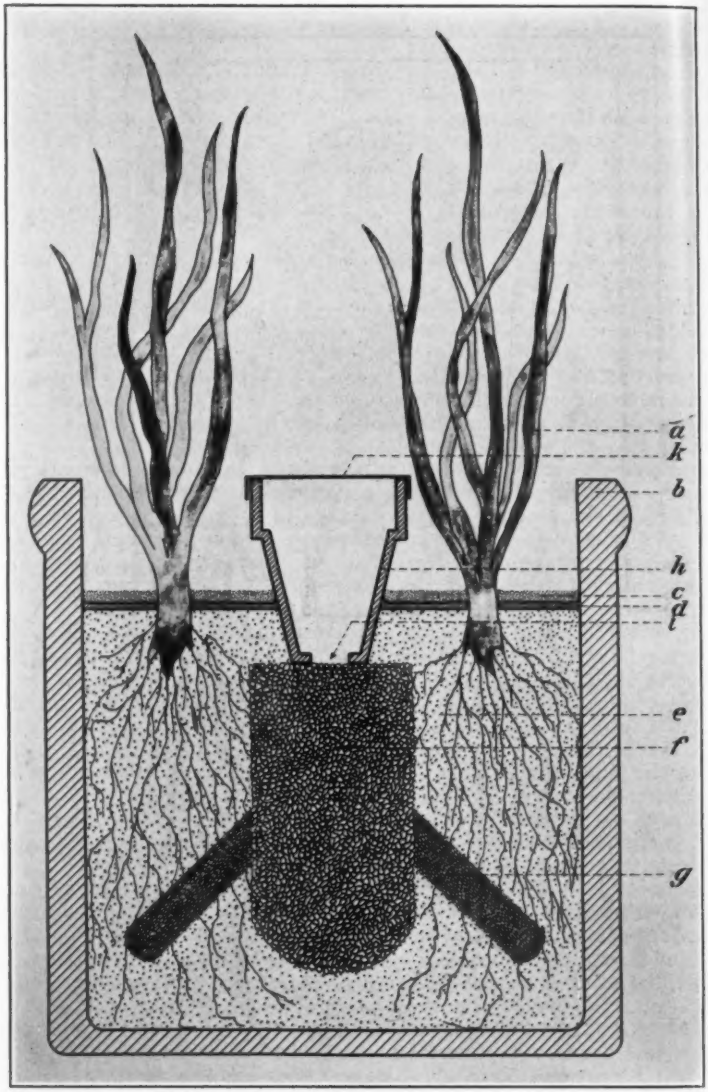

FIGURE 1.—Schematic drawing of vertical cross section through a container of the type used in the experiments with spring wheats: *a*, Plant; *b*, glazed stone jar; *c*, layer of leached sand; *d*, wax seal; *e*, soil; *f*, coarse-gravel central core; *g*, coarse-sand side core; *h*, small flowerpot through which soil was watered; *i*, enlarged opening in bottom of flowerpot; *k*, half of a small Petri dish over top of flowerpot.

brought back to their original weight by the addition of water, as necessity required. While the plants were very small, this was necessary only once every 4 or 5 days. From the time the plants were in the 4-leaf or 5-leaf stage until they began active jointing, they required watering every second day, and thereafter, especially during bright, warm weather, they frequently required watering every day. Because of the small mass of soil in the jars, it was found necessary, in both seasons after the plants were half-grown, to increase the water content in order to prevent wilting and maintain normal continuation of growth.

A solution balance proved to be sufficiently accurate for all weighings. At each weighing, after the loss of weight was recorded, the balances were set at the original weight of the jar and tap water was added by means of a rubber tube connected to an overhead reservoir, until the scales balanced.

At various times during the course of the experiments, one of the jars was dismantled to ascertain the distribution of plant roots and soil moisture. Although it was found that the greatest concentration of roots was against the sides and bottom of the jar, as is nearly always the case when plants are grown in small containers, there was excellent distribution of roots throughout the soil mass. The distribution of moisture was also satisfactory in all jars examined.

It was desired to learn the effect of leaf-rust infection on yield, growth characters, and water economy, when infection was initiated at various stages of growth. Each variety, therefore, was divided into 5 groups of 6 jars each, each jar containing 6 plants. One group of each variety was maintained to maturity as a rust-free control. The remaining 4 groups of each variety were first inoculated with leaf rust at the seedling, jointing, booting, and flowering stages, respectively. After the first inoculation, each inoculated group was reinoculated frequently enough to keep all leaves of the susceptible Pusa No. 4 abundantly rusted and all leaves of the resistant Warden copiously flecked.

When the plants of each variety had reached their full stature they were measured for height, and the number of tillers and heads was recorded. The dates of heading and approximate dates of maturity also were recorded for each jar of each group.

As each group reached full maturity the heads were harvested, the stems were cut off at the crown just below the soil level, and the roots were washed out of the soil. The heads were threshed and the chaff added to the harvested tops which were cut up and dried in an electric oven for 48 hours at 100° C. and then weighed; these parts have been designated as "straw." The seed was air-dried for several weeks, then weighed. The roots were washed free from soil and trash as thoroughly as possible, after which they were dried in the electric oven at 100° and weighed. All determinations on yields and all calculations on water requirement were, therefore, based on the weights of thoroughly dried materials. Total dry matter was determined by adding the weights of straw, grain, and roots together.

In calculating the weights for total dry matter all recoverable plant parts, including roots, were used. The yields for each class of plant material and each rust period represent the total weights from 6 jars containing 6 plants each. Thus the weights for each year represent the totals for 36 plants in each category.

EXPERIMENTAL RESULTS

The results from the experiments with spring wheat varieties in 1931–32 and 1932–33 were so consistent and convincing that it was considered advisable to terminate the experiments at the end of the second greenhouse year. The detailed data on water losses, yields, growth characters, and water requirements are presented in table 1 and the averages of these data for the 2-year period in table 2.

TABLE 1.—*Effect of leaf-rust infection, initiated at various stages of growth, on the yield, growth characters, and water requirement of a susceptible and a resistant spring wheat grown in the greenhouse at Manhattan, Kans., 1931–32 and 1932–33*

Year of experiment and stage of growth at first inoculation [1]	Total dry weight				Total number			Average height of plants	Days required to—		Total water used	Water requirement based on weight of—		Average rust infection
	Dry matter	Straw	Roots	Grain	Tillers	Heads	Kernels		Head	Mature		Total dry matter	Grain	
PUSA NO. 4, C. I. 8899 (SUSCEPTIBLE)														
1931-32	*Grams*	*Grams*	*Grams*	*Grams*				*Mm*	*Number*	*Number*	*Grams*	*Grams*	*Grams*	*Percent*
Seedling	90.8	77.7	4.2	8.9	134	74	276	483	83	151	82,200	905.3	9,235.9	84.5
Jointing	105.4	89.2	4.6	11.6	143	92	322	554	71	140	91,685	869.9	7,903.8	85.0
Booting	125.6	100.6	4.5	20.7	129	88	551	641	66	132	99,405	791.4	4,802.2	84.6
Flowering	168.5	116.0	5.1	46.9	142	96	1,144	724	65	130	104,315	619.1	2,224.2	84.1
Control	228.3	127.5	18.0	82.8	167	102	1,658	775	65	129	104,295	456.8	1,259.6	.0
1932-33														
Seedling	103.4	96.1	5.2	2.1	205	91	56	396	71	146	135,973	1,315.0	64,749.0	85.0
Jointing	135.1	113.1	7.1	14.9	209	135	486	454	64	140	148,624	1,100.1	9,974.7	85.0
Booting	132.5	110.8	6.8	14.9	199	118	680	472	61	130	149,987	1,131.9	10,066.2	85.0
Flowering	183.5	121.0	5.7	56.8	179	161	1,904	574	61	127	149,432	814.3	2,630.8	85.0
Control	246.1	137.5	11.4	97.2	179	169	2,345	611	60	125	155,375	631.3	1,598.5	.0
WARDEN, C. I. 4994 (RESISTANT)														
1931-32														
Seedling	527.2	362.8	70.9	93.5	298	195	2,978	730	142	200	264,020	500.8	2,823.7	2.1
Jointing	532.1	373.4	75.0	83.7	250	163	2,738	760	140	198	260,735	490.0	3,115.1	2.1
Booting	526.1	369.0	53.7	103.4	237	155	2,792	820	139	192	267,966	509.3	2,591.5	1.9
Flowering	508.3	355.6	58.2	94.5	222	146	2,528	780	137	190	244,375	480.7	2,586.0	2.0
Control	517.5	364.1	47.9	105.5	246	150	2,838	800	140	185	264,370	510.8	2,505.8	.0
1932-33														
Seedling	476.0	348.9	40.1	87.0	306	199	2,632	735	157	202	295,144	620.0	3,392.4	1.4
Jointing	482.1	350.8	44.7	86.6	287	195	2,638	711	154	200	291,593	604.8	3,367.1	1.4
Booting	509.1	369.3	40.3	99.5	276	198	2,874	771	151	195	301,342	591.9	3,028.5	1.5
Flowering	508.9	371.8	37.7	99.2	273	204	2,857	775	149	194	305,301	599.9	3,077.6	1.5
Control	513.6	378.7	39.5	95.4	268	193	2,559	765	150	192	303,811	591.5	3,184.6	.0

[1] There were 36 plants in each group of each variety.

TABLE 2.—*Averages of the various data on the effect of leaf rust on the yield, growth characters, and water requirement of a susceptible and a resistant variety of spring wheat for 1931–32 and 1932–33 at Manhattan, Kans.*

Stage of growth at first inoculation [1]	Average dry weight				Average			Average height of plants	Average days required to—		Average water used	Average water requirement based on weight of—		Average rust infection
	Dry matter	Straw	Roots	Grain	Tillers	Heads	Kernels		Head	Mature		Total dry matter	Grain	
	Grams	*Grams*	*Grams*	*Grams*	*No.*	*No.*	*No.*	*Mm*	*No.*	*No.*	*Grams*	*Grams*	*Grams*	*Percent*
PUSA NO. 4 (SUSCEPTIBLE)														
Seedling	97.1	86.9	4.7	5.5	169	82	166	439	77	148	109,086	1,110.1	36,992.4	84.7
Jointing	120.2	101.1	5.9	13.2	176	113	404	504	67	140	120,154	985.0	8,939.2	85.0
Booting	129.0	105.7	5.6	17.8	164	103	615	556	63	131	124,696	961.6	7,434.2	84.8
Flowering	176.0	118.5	5.4	51.8	160	128	1,524	649	63	128	126,873	716.7	2,427.5	84.5
Control	237.2	132.5	14.7	90.0	173	135	2,001	693	62	127	129,835	544.0	1,429.0	.0
WARDEN (RESISTANT)														
Seedling	501.6	355.8	55.5	90.2	302	197	2,805	732	149	201	279,582	560.4	3,108.0	1.7
Jointing	507.1	362.1	59.8	85.1	268	179	2,688	735	147	199	276,164	547.4	3,241.1	1.7
Booting	517.6	369.1	47.0	101.4	256	176	2,833	705	145	193	284,654	550.6	2,810.0	1.7
Flowering	508.6	363.7	47.9	96.8	247	175	2,692	777	143	192	274,838	540.3	2,831.8	1.7
Control	515.5	371.4	43.7	100.4	257	171	2,698	782	145	188	284,060	551.1	2,845.2	.0

[1] There was an average of 36 plants in each group of each variety.

These data show that leaf rust on the susceptible variety reduced the yield of straw, grain, and roots, and the number of heads and kernels, and increased the water requirement and the number of days necessary for it to head and mature. The data also show that the effect was proportional to the length of the infection period rather than to the amount of rust, and further demonstrate that the rust had comparatively little effect on the resistant variety. It is worthy of note that the response of the plants to leaf-rust infection was relatively the same in the 2 separate years, although the actual measurements varied in some cases as would be expected.

Effect of Leaf-Rust Infection on Yield of Grain and Other Plant Parts

In table 3 is shown the effect of leaf-rust infection initiated at various stages of growth on the average yield of total dry matter, straw, grain, and roots of two varieties of spring wheat, expressed in percentage increase or decrease from the control plants.

Table 3.—*Effect of leaf-rust infection, initiated at various stages of growth, on the average yield of total dry matter, straw, grain, and roots of 2 varieties of spring wheat for 1931–32 and 1932–33*

[Expressed as percentage increase or decrease from the control plants]

Variety and stage of growth at first inoculation	Increase or decrease in indicated plant part			
	Total dry matter	Straw	Grain	Roots
Pusa No. 4 (susceptible):	*Percent*	*Percent*	*Percent*	*Percent*
Seedling	−59.0	−34.4	−93.8	−68.0
Jointing	−49.3	−23.6	−85.3	−59.8
Booting	−45.5	−20.2	−80.2	−61.9
Flowering	−25.8	−10.5	−42.4	−63.2
Warden (resistant):				
Seedling	−2.6	−4.2	−10.1	+27.0
Jointing	−1.6	−2.5	−15.2	+36.8
Booting	+.4	−.6	+1.0	+7.5
Flowering	−1.3	−2.0	−3.5	+9.6

The data show that the average yield of total dry matter of susceptible Pusa No. 4 was greatly reduced when the plants were heavily rusted from the seedling stage to maturity. When rust infection did not occur until the flowering stage, however, the reduction was less than half as much. In the resistant Warden the reduction at all stages was negligible and probably within the experimental error.

The yield of tops or straw was less affected by leaf-rust infection than any of the other plant parts in susceptible Pusa No. 4. In this case also the reductions were smaller with each reduction in the length of the rust period. In the resistant Warden the reductions in straw weight caused by leaf rust were small and in no way comparable to those occurring in the susceptible variety.

The greatest reduction in yield of plant material caused by leaf rust occurred in the yield of grain of the susceptible Pusa No. 4. The results are well illustrated in figure 2. The yields for Pusa No. 4 in 1931–32 are somewhat nearer the expected results than are those for 1933 or the averages for the 2-year period. This is due primarily to two factors: (1) The rust infection in the early inoculated group in

1932–33 was so heavy that grain production was nearly inhibited, and (2) the boot-stage-inoculation group, in 1932–33, developed a very heavy infection on the peduncles, which caused rather severe shriveling of the seed. The weight of grain, consequently, was the same as that produced by the group rusted from jointing to maturity. This unusual and very marked effect of shriveling because of leaf-rust infection on the peduncles is well illustrated by comparing the yields with the kernel numbers given in table 1. The number of kernels in the boot-stage-inoculation group of Pusa No. 4 is higher than that for the jointing-stage-inoculation group, although the weight of grain in grams for the two groups is the same.

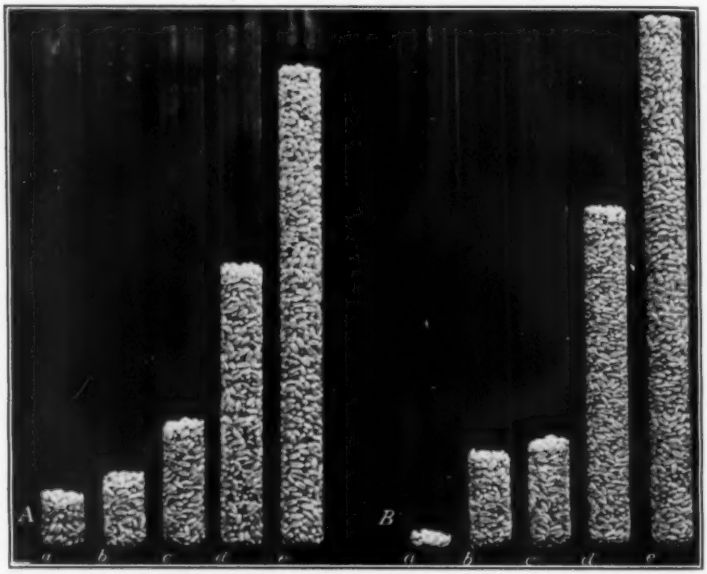

FIGURE 2.—Total yield of grain from 5 groups of 36 plants each of Pusa No. 4 grown in sealed containers in the greenhouse at Manhattan, Kans. *A* in 1931–32 and *B* in 1932–33: *a*, *b*, *c*, and *d*, Yield of grain from groups in which leaf-rust infection was initiated in the seedling, jointing, booting, and flowering stages, respectively; *e*, yield of grain from uninoculated control group.

The marked reduction in yield of grain of the susceptible variety caused by leaf rust is well illustrated by the percentages calculated on the average yield for the 2-year period shown in table 3. Because of several factors, these reductions are undoubtedly much greater than those occurring under field conditions. The limitation in soil mass and hence in feeding area, because of growing the plants in closed containers, undoubtedly is one of the prime factors. Furthermore, the rust infection was considerably heavier than that usually occurring in the field. In these experiments, after a group of plants was given its first inoculation it was reinoculated at short intervals to keep all leaves heavily infected. These frequent reinoculations caused not only all leaves but also all parts of each leaf, as well as the leaf sheath, to become heavily infected. In the field individual leaves may have varying amounts of rust, and only parts of some

leaves are rusted, while the leaf sheaths frequently show little or no infection. It seems certain, therefore, that the results obtained in these experiments represent a nearly maximum effect.

It has already been mentioned that the reduction in yield for the boot-stage-inoculation group of Pusa No. 4 in 1932–33 was too great; consequently, the average yield probably is too low. A more reasonable figure probably is obtained in this case by using the yields for that group in 1932. It is of interest to note that in 1932 the yield of the boot-stage-inoculation group was 75.0 percent below that of the control group, while the flowering-stage-inoculation group was 43.3 percent below that of the control. These figures are of much interest and significance because they illustrate how much greater is the effect of leaf rust on yield when heavy infection develops rather early in the spring than when it develops late. Leaf-rust infection usually does not reach its maximum development in the Great Plains area until about flowering time, but in wet seasons it may become very abundant while wheat is still in the boot. These experiments indicate that, if the rust attack is severe as early as the booting stage, the loss in yield may be nearly twice as great as when it does not become severe until flowering time.

The data on the reduction in yield of grain because of leaf-rust infection on resistant Warden do not present quite so convincing a picture as those for Pusa No. 4 (fig. 3). There is considerable fluctuation among the various groups, probably because of a combination of small numbers and the relatively slight effect of the infection. For example, a reduction of 10.1 percent was obtained in the average yield of the group rusted from the seedling stage to maturity, while a 15.2 percent reduction was obtained in the group rusted from the jointing stage to maturity. In contrast to this there was an apparent increase of 1.0 percent in the yield of the group rusted from the booting stage to maturity, while the group rusted from flowering to maturity showed an average yield 3.5 percent below that of the controls.

Despite these discrepancies, however, there is evidence that leaf-rust infection does cause some loss in grain yield, even in a highly resistant variety, and that this loss is greatest when infection occurs very early. This seems entirely logical in view of the fact that leaf rust nearly always produces flecking in resistant wheat varieties. In these experiments flecking was extremely heavy in the inoculated groups because of frequent reinoculations. Flecking also is frequently abundant when there is an abundance of inoculum under field conditions. There can be little doubt, therefore, that abundant flecking was responsible for the yield reductions noted in the inoculated groups of Warden.

The maximum occurrence of uredia on the leaves of Pusa No. 4 was about 85 percent each year, while a maximum infection of only about 2 percent in the form of uredia occurred each year on the inoculated groups of Warden. Furthermore, it should be kept in mind that the pustules on Warden were minute and confined almost entirely to the lower leaves. Small uredia developed very sparsely on the lower leaves at about the time they began to turn yellow and become senescent. This probably was because of the long incubation period of the fungus on the highly resistant variety. Instead of uredia heavy flecking was produced on the more active upper leaves. This naturally reduced the photosynthetic area, but was not accompanied by an

active feeding of the fungus on elaborated plant food as is the case in a susceptible variety such as Pusa No. 4. The observed reductions in yield because of leaf-rust infection on the resistant variety would, therefore, seem to be very reasonable and in agreement with the expected results. They indicate clearly that there is a small but definite loss in grain yield because of heavy leaf-rust infection, even on a variety as highly resistant as Warden.

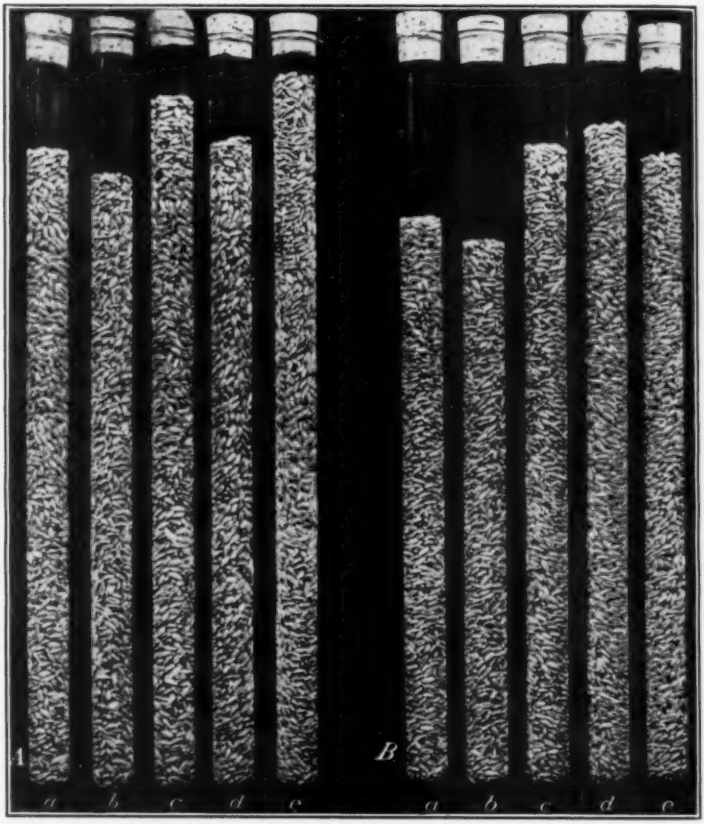

FIGURE 3.—Total yield of grain from 5 groups of 36 plants each of Warden grown in sealed containers in the greenhouse at Manhattan, Kans., *A* in 1931–32 and *B* in 1932–33: *a*, *b*, *c*, and *d*, Yield of grain from groups in which leaf-rust infection was initiated in the seedling, jointing, booting, and flowering stages, respectively; *e*, yield of grain from uninoculated control group.

Perhaps the most striking effect of leaf-rust infection on the wheat plant is expressed on the roots. The marked effect observed in these experiments is well represented in the weights of roots from the various groups of Pusa No. 4 presented in table 1. The weight of recoverable roots was about the same for all the rusted groups and was only about one-fourth of that of the control group. The average weights of roots in the seedling-, jointing-, booting-, and flowering-stage-inoculation

groups of Pusa No. 4 were lower than the average weight for the rust-free control, as shown in table 3. In the case of the resistant Warden, however, no reduction in weight of roots was observed; in fact, the weights for the rusted groups nearly always exceeded the weight of the control.

The differences in the weights of roots recovered from the inoculated and control groups of Pusa No. 4 were no greater than the differences in the visible appearance of the roots. Although great care was used in washing the roots free from the soil and every effort was made to recover all of them, it was impossible to prevent the loss of a large proportion of the fine fibrous roots. This was particularly true in the inoculated groups, where it was found that most of the small roots were dead or dying. These roots were dark brown and fell to pieces in the

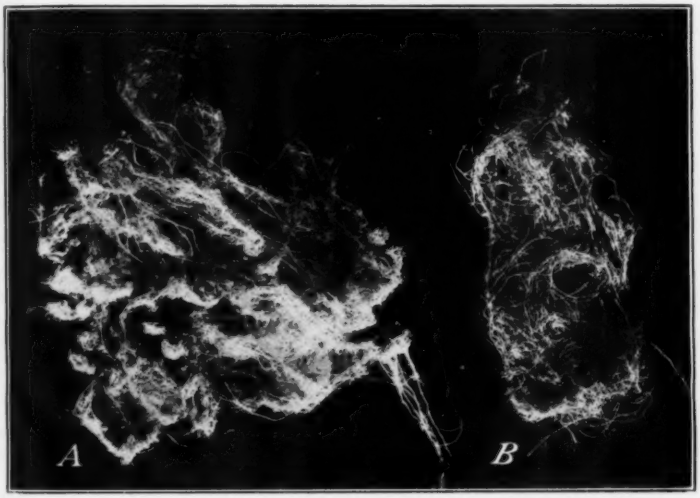

FIGURE 4.—Roots of Pusa No. 4 from two sealed containers, showing the effect of leaf-rust infection on the weight, number, and quality of the roots: *A*, Roots from 6 uninoculated plants; *B*, roots from 6 plants in which infection was initiated at the booting stage.

process of washing. Hence, about all that could be recovered from those groups were the coarser main roots, and even those were badly discolored. The root systems recovered from the control jars retained many of the small fibrous roots and all were a normal healthy yellow. These differences are shown in figure 4.

The results of these experiments indicate that heavy leaf-rust infection of a susceptible variety of wheat has a serious effect on the root system. The shock of sudden heavy rust development on the leaves apparently is transmitted almost immediately to the roots and results in their rapid deterioration. The effect seems to be about the same regardless of the length of association of the host and parasite. The roots of plants that were rust-free until flowering were nearly as severely injured as those of plants rusted from the seedling stage to maturity. In the former case an extensive root system developed but was greatly reduced by rust infection, whereas in the latter case the

root system never became so extensive as in the former and those roots that did develop were unable to persist long in a healthy condition.

Effect of Leaf-Rust Infection on Number of Tillers, Heads, and Kernels

The fact that marked reductions in the yield of a susceptible variety result from heavy infections of leaf rust having been established, the question arises as to whether these losses are due to decreases in number or in size of the parts concerned. The data in table 1 show the number of tillers, heads, and kernels produced in each group of each variety in each of the 2 years, and in table 2 are given the average numbers for the 2-year period. These data clearly show that the reduction in number of kernels is proportionately greater than the reduction in the number of heads and tillers. The number of kernels produced would, therefore, seem to be a very important point. These relations are perhaps more strikingly shown in table 4, in which the reduction is expressed as a percentage of the controls. The average number of kernels in each of the groups first inoculated in the seedling, jointing, booting, and flowering stages was lower than that of the control. On the other hand the percentage reduction in number of heads was less—usually much less—than half as much, and the number of tillers was scarcely reduced at all.

Table 4.—*Effect of leaf-rust infection, initiated at various stages of growth, on the average number of tillers, heads, and kernels of two varieties of spring wheat for 1931–32 and 1932–33*

[Expressed as percentage increase or decrease from the control plants]

Variety and stage of growth at first inoculation	Increase or decrease in indicated plant part			Variety and stage of growth at first inoculation	Increase or decrease in indicated plant part		
	Tillers	Heads	Kernels		Tillers	Heads	Kernels
Pusa No. 4 (susceptible):	*Percent*	*Percent*	*Percent*	Warden (resistant):	*Percent*	*Percent*	*Percent*
Seedling	−2.3	−39.2	−91.7	Seedling	+17.5	+15.2	+3.9
Jointing	+1.7	−16.2	−79.8	Jointing	+4.2	+4.6	−.4
Booting	−5.2	−23.7	−69.2	Booting	−.4	+2.9	+5.0
Flowering	−7.5	−5.1	−23.8	Flowering	−3.8	+2.3	−.2

There was practically no reduction in the number of the various plant parts noted in the inoculated plants of the resistant variety; in fact, increases were encountered in most instances. With the exception of the increase in the number of heads and tillers on plants rusted from the seedling stage to maturity, the increases were small and probably within the experimental error.

There was an increase in number of late-developing tillers in severely infected plants. This phenomenon was particularly marked in 1932–33 and reached its best expression in the susceptible variety (table 1). In late spring, when leaf rust was extremely heavy on the earlier inoculated groups and much of the leaf tissue had been killed, many new tillers were put out by this variety. The increase in average number of tillers was greater in Warden, but in that case the extra tillers appeared much earlier. The production of supernumerary tillers in Pusa No. 4 after the plants were mature was of much interest, for this variety is one of low yielding capacity because of its extreme earliness and limited tillering ability. It has frequently been noted

that when the wheat plant is cut off or mechanically broken down after it has headed but has not reached maturity, it may put out many new tillers. Heavy leaf-rust infection, which prematurely kills the leaves and other vegetative parts and prevents normal maturity, may bring about the same reaction.

The weight per 100 kernels, based on the total number of kernels produced, was calculated for the susceptible Pusa No. 4. The reductions were 26.6, 28.8, 35.5, and 24.4 percent, respectively, in the seedling-, jointing-, booting-, and flowering-inoculation groups. When the same calculations were made for the resistant variety, however, it was found that the weight per 100 kernels in the four inoculated groups was reduced 13.5, 16.1, 5.4, and 5.4 percent, respectively. These results are similar to those obtained by Caldwell and his associates (*2*).

As a general rule the reduction in kernel weight caused by leaf rust is due, not to shriveling, but principally to the smaller size of the kernels. Shriveling is a difficult character to measure except where wide differences exist, and slight visible differences, therefore, are hard to evaluate. Except in those rare instances when leaf rust develops on the peduncles, there is little noticeable increase in shriveling in seed from a leaf-rust-infected crop. There was little visible difference in shriveling of the seed among the various groups of the two varieties of wheat used in these experiments, with the exception of the one group of Pusa No. 4, in 1932–33, in which a heavy leaf-rust infection developed on the peduncles shortly after emergence of the heads. Actual counts of the number of pinched kernels in other groups of both varieties yielded no very marked evidence of shriveling caused by leaf rust. This agrees in general with the impression gained from casual visual examination of the seed.

Leaf rust reduced the number of heads in the inoculated groups of Pusa No. 4, as shown in table 4. These data would seem to indicate that if reduction in the number of heads is important it is considerably less important than the reduction in the number of kernels. The size of the heads of a susceptible variety also is materially reduced, as shown in figure 5. Heads on badly rusted plants seldom emerged fully from the boot and were pinched in appearance.

Figure 5 also shows clearly the effect of leaf rust on the setting of grain in the spikelets. In the control group all spikelets from the base to the tip set grain in most instances. Nearly all spikelets had 2 kernels and some had 3. In the rusted group only an occasional spikelet developed grain and these were usually toward either the middle or base of the spike. Most spikelets developing grain had only 1 kernel, but this one was rather plump.

Effect of Leaf-rust Infection on Height of Plant, Time of Heading, and Time of Maturity

One of the striking effects of leaf rust is the reduction in height of the plant. This is clearly shown by the measurements given in tables 1, 2, and 5, and in figures 6 and 7. As shown in table 2, the average height of plants of Pusa No. 4 for the 2-year period was gradually reduced from 693 mm in the control group to 439 mm in the group rusted from the seedling stage to maturity, a maximum reduction of 254 mm, or 36.6 percent (table 5). In the resistant variety, however,

the maximum reduction in average height of plant was only 50 mm, or 6.3 percent. Thus, the effect of leaf rust on height of plant apparently is very slight in a resistant variety, regardless of the stage of growth at which infection first occurs (fig. 7). In a susceptible variety the data indicate that plant height is only slightly reduced when leaf-rust infection occurs late in the spring, but may be reduced about one-third when infection develops very early in the life of the plant.

Another pronounced effect of leaf-rust infection is the prolongation of the time required to reach full heading. Thus (table 2) Pusa No. 4 plants, first inoculated in the seedling stage, required on an average

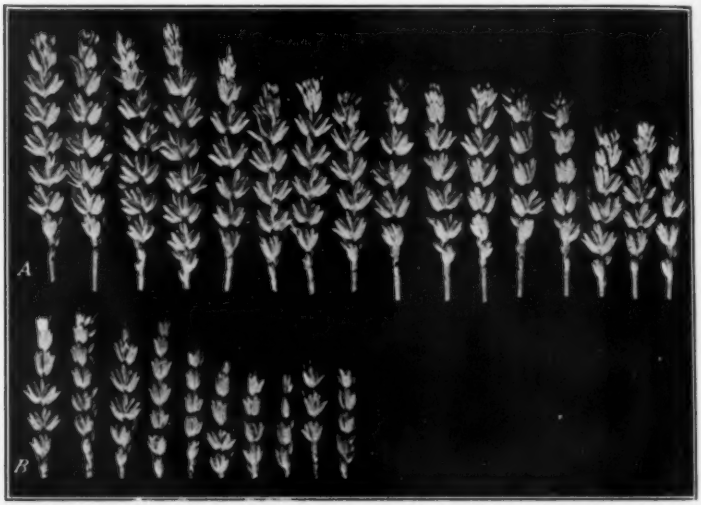

FIGURE 5.—Heads of Pusa No. 4 from two sealed containers, showing the effect of leaf-rust infection on the number and size of the heads produced: *A*, Total number of heads from six uninoculated plants; *B*, total number of heads from six plants in which infection was initiated at the seedling stage.

15 days more to reach heading than did the nonrusted controls. Heading in the jointing-stage-inoculation group was retarded an average of 5 days and in the booting- and flowering-stage-inoculation groups the retardation averaged 1 day. The percentage differences as shown in table 5 varied from 1.6 to 24.2 percent, depending on time of initiation of infection. In the case of the resistant variety, however, the greatest retardation of the average number of days required to head was 4 days, or 2.7 percent, recorded for the seedling-stage-inoculation groups. These data indicate that heavy leaf-rust infection, developing early in the season, definitely retards heading in a susceptible variety but only very slightly retards that of a resistant one.

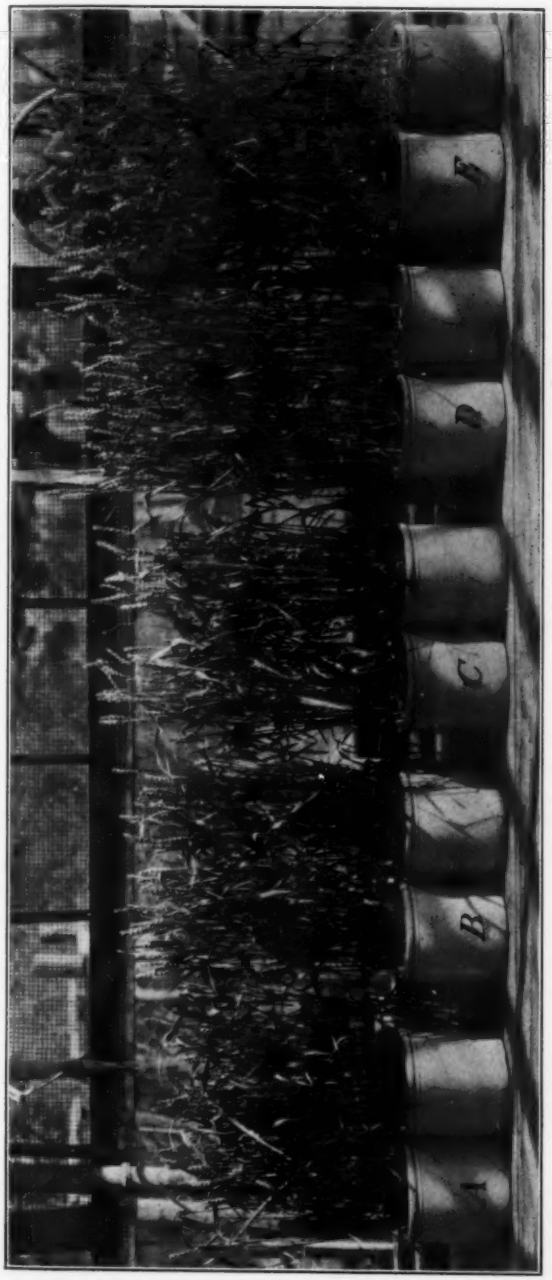

FIGURE 6.—General appearance near the time of plant maturity of Pusa No. 4 in which leaf-rust infection had been initiated at various stages of development: *A*, *B*, *C*, *D*, Groups in which infection was initiated at the seedling, jointing, booting, and flowering stages, respectively; *E*, uninoculated control group.

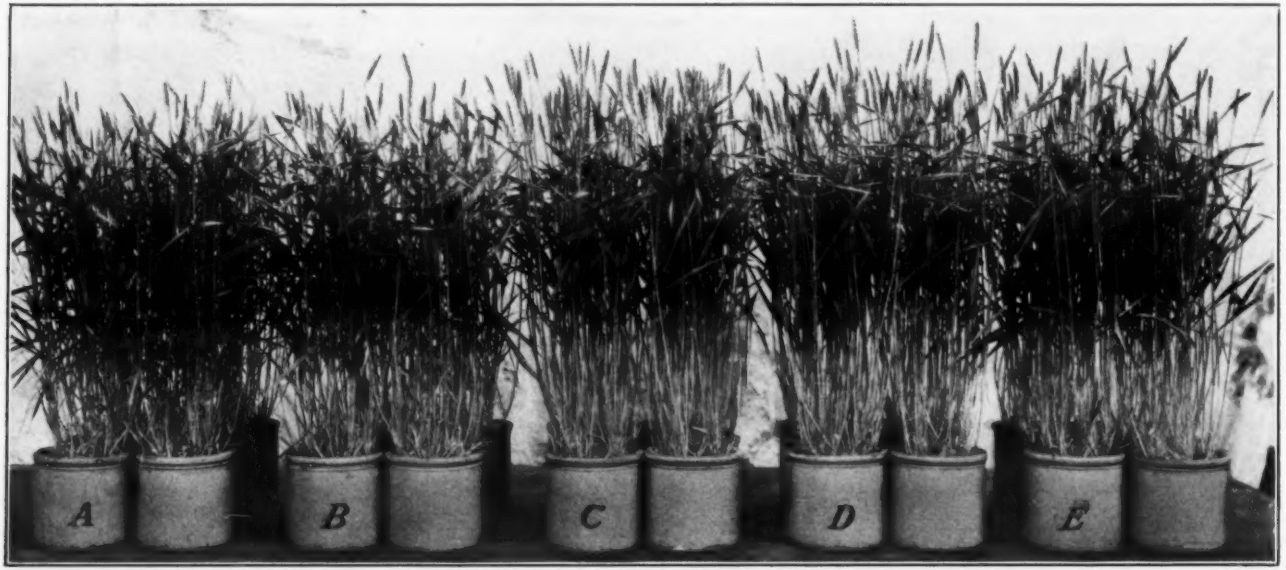

FIGURE 7.—General appearance near the time of plant maturity of Warden in which leaf-rust infection had been initiated at various stages of development: *A*, *B*, *C*, *D*, Groups in which infection was initiated at the seedling, jointing, booting, and flowering stages, respectively; *E*, uninoculated control group.

TABLE 5.—*Effect of leaf-rust infection, initiated at various stages of growth, on the average height of plant and on the number of days required to head and to mature in two spring wheat varieties for 1931–32 and 1932–33*

[Expressed as percentage increase or decrease from the control plants]

Variety and stage of growth at first inoculation	Increase or decrease in—			Variety and stage of growth at first inoculation	Increase or decrease in—		
	Height of plant	Days to head	Days to mature		Height of plant	Days to head	Days to mature
Pusa No. 4 (susceptible):	*Percent*	*Percent*	*Percent*	Warden (resistant):	*Percent*	*Percent*	*Percent*
Seedling	−36.6	+24.2	+16.5	Seedling	−6.3	+2.7	+6.9
Jointing	−27.2	+8.1	+10.2	Jointing	−6.0	+1.3	+5.8
Booting	−19.7	+1.6	+3.1	Booting	+1.6	.0	+2.6
Flowering	−6.3	+1.6	+.8	Flowering	−.6	−1.3	+2.1

The time required to reach maturity is also considerably lengthened, as would be expected, the increases depending on time of inoculation.

The lengthening of the maturation period is due in part to the lengthening of the time required by the rusted plants to come to head and also by the extension of the fruiting period itself. For example, the control groups of Pusa No. 4 required an average of 65 days from heading to maturity, whereas in the seedling-stage-inoculation group the time from heading to maturity was 71 days—a difference of 6 days. For Warden the fruiting period of the same rusted group was 9 days greater than the average of the controls, a much longer time than that required to reach heading.

The visible results of heavy rust infection on the susceptible variety were very noticeable. Heads of copiously rusted plants emerged very slowly; many of them never fully cleared the boot. After emergence the peduncles on such plants lengthened very slowly or not at all and their maximum extension was much less than that in the controls (fig. 5).

Another striking reaction of the susceptible plants was their failure to turn a normal yellow on ripening. They remained a grayish green, even after the kernels had become quite hard; hence, it was very difficult to determine accurately when the plants were fully mature. It could be done accurately only by an examination of the seed. Leaf sheaths and glumes often remained green and active after kernel maturity.

EFFECT OF LEAF-RUST INFECTION ON WATER ECONOMY OF THE WHEAT PLANT

The third phase of the studies constituting the investigations herein discussed consisted of experiments on the relation of leaf-rust infection to the water economy of a susceptible and a resistant variety. Inasmuch as economy in the use of water by plants probably can be best measured by their water requirements, the discussion and data presented in this paper are expressed in those terms. The term "water requirement" is herein employed to express the number of units of water used by the plant in the production of one unit of dry matter. The plants used were the same as those employed in the other phases of the experiments considered in the foregoing discussion. They were grown to maturity in sealed stone jars and a careful record was kept of the quantity of water used during their lifetime. The apparatus

and methods of procedure are fully described under Materials and Methods.

The efficiency of the apparatus used was somewhat roughly measured by blank jars, which were set up exactly as were those containing plants but in which no plants were grown. There were 5 of these jars in 1931–32, but only 2 were available for use in 1932–33. One of them became water-logged through error and was discarded, so only 1 was used as a blank during the season. The blank jars were run 202 days each year, and lost a total of 1,420 g of water in 1931–32 and 785 g in 1932–33. This is an average loss of 1.4 g and 3.8 g per jar per day for the 2 separate years. Although considerably more water was lost through the apparatus in 1932–33 than in 1931–32, the total quantity was still very small when compared with that lost from jars containing plants. The average daily water loss per jar for the latter was 164.7 g in 1931–32 and 225.8 g in 1932–33. Thus, the daily water loss, because of imperfections in the apparatus, was 0.8 percent of the average daily loss from jars containing plants in 1931–32 and 1.6 percent in 1932–33. This gives an average of 1.3 percent error through leakage in the apparatus for the 2-year period, which is a very small percentage when the length of time that the experiments were in progress is considered.

The data in table 1 show that much more water was used in 1932–33 than in 1931–32. This was principally due to the fact that the fall and winter of 1932–33 were very mild, marked by long periods of days when the sun shone and greenhouse temperatures were relatively high with a consequent increase in transpiration.

Although considerably more water was used in 1932–33 than in 1931–32, correspondingly high increases in the yield of plant materials did not occur. The total dry matter and the dry weight of tops (straw) were somewhat higher in 1932–33 than in 1931–32, but the differences in the weight of roots and grain were very small.

It will be noted (table 2) that Warden required slightly more than twice as much water as Pusa No. 4. This is to be expected, since Warden matures much later and is vegetatively more prolific than Pusa No. 4. Warden grew a minimum of 56 days longer than Pusa No. 4 in 1932 and 67 days longer in 1933. It also had more tillers, heads, and leaves, and was taller and produced more grain than Pusa No. 4 in each of the 2 years.

The water requirement was calculated on two separate bases, viz, weight of total dry matter and dry weight of grain. Considering the data for both years, the total dry matter seems to be the best basis for calculating this factor. The average of the water requirement (table 2) of Pusa No. 4, for the groups first inoculated with rust in the seedling, jointing, booting, and flowering stages, was much greater than for the control group. These data (table 6) show clearly that the longer the association of the host and parasite, the greater is the increase in the water requirement.

TABLE 6.—*Effect of leaf-rust infection, initiated at various stages of growth, on the water requirement, calculated on the basis of total dry matter and of dry weight of grain, of two varieties of spring wheat for 1931–32 and 1932–33*

[Expressed as percentage increase or decrease from the control plants]

Variety and stage of growth at first inoculation	Increase or decrease in water requirement of plants calculated on basis of—		Variety and stage of growth at first inoculation	Increase or decrease in water requirement of plants calculated on basis of—	
	Total dry matter	Dry weight of grain		Total dry matter	Dry weight of grain
Pusa No. 4 (susceptible):	*Percent*	*Percent*	Warden (resistant):	*Percent*	*Percent*
Seedling	+104.0	+2,488.6	Seedling	+1.6	+9.2
Jointing	+81.0	+525.5	Jointing	−.7	+13.9
Booting	+76.7	+420.2	Booting	−.1	−1.2
Flowering	+31.7	+69.8	Flowering	−1.9	−.5

The only group of the resistant Warden that shows a greater water requirement than the rust-free control group is the one infected from the seedling stage to maturity. The increase is only 1.6 percent and almost certainly is not significant.

Although water requirements calculated on the basis of the weight of total dry matter seem more reliable, some investigators have calculated them on the basis of grain weight alone. In the experiments herein reported water requirements were calculated by both methods as shown by the data in tables 1, 2, and 6. When calculations were based on grain weight the water requirements were very high for all groups of the susceptible variety. The increase recorded (table 6) for the seedling-stage-inoculation group of Pusa No. 4 was especially high in 1933. This was due not so much to a greater use of water as to the production of an extremely small quantity of grain.

In the case of the resistant Warden the water requirements figured on the basis of dry weight of grain showed increases for the seedling-stage- and jointing-stage-inoculation groups, respectively, whereas the booting-stage- and flowering-stage-inoculation groups were below those for the control group. While the percentages for the two groups show increases that are higher than those obtained when the total dry matter was used as the basis for calculation, they are extremely small as compared with those for the susceptible variety.

The experiments seem to prove beyond question that heavy infections of leaf rust cause great increases in the water requirement of a susceptible variety of wheat and a very slight increase or none in the case of a resistant variety. The total amount of water used by a susceptible variety when the plants are infected for some time before maturity is considerably smaller than that used by nonrusted plants or those rusted for short periods only. This is to be expected when it is considered that heavy and prolonged leaf-rust infection of such a variety results in reduction of vegetative material and hastens the death of many leaves. This, combined with reductions in the number and size of leaves, naturally greatly reduces the transpiring area of the plants.

DISCUSSION

It seems advisable to emphasize here some of the principal results of the studies reported in this paper and to compare them with the conclusions of other investigators. The fact that leaf-rust infection on wheat causes reduction in yield of grain has been amply proved by Mains (*13*), Neill (*15*), Johnston (*8*), and Caldwell et al. (*2*), but there is some diversity of opinion as to the way in which the losses are expressed. Mains, Johnston, and Caldwell report that the reductions in yield are caused primarily by a reduction in the number of kernels per head and secondarily by a reduction in kernel size. Neill reports, however, that losses are principally due to a reduction in weight of individual grains and, to a much smaller extent, to the production of fewer kernels per head. In the experiments reported here, the weights of individual kernels from rusted plants of a susceptible variety were 24.4 to 35.5 percent less than those from rust-free plants of a given variety, depending on the stage of growth at which infection was initiated. The number of kernels per head in the same material varied from 23.8 to 91.7 percent below that for the rust-free controls. It seems clear, therefore, that when soil moisture is ample, as it was in these experiments, the yield reductions caused by leaf rust are brought about principally by the production of fewer kernels per head and to a lesser extent by a reduction in the weight of the individual kernels. The data obtained indicate that the estimate by Caldwell and his associates (*2*), to the effect that about 75 percent of the reduction in yield was due to the production of fewer kernels per head and about 25 percent to a smaller kernel size, is reasonable and probably about correct. When leaf-rust infection develops on the peduncles of the wheat heads, as occurred in the booting-stage-inoculation group of Pusa No. 4 in 1932–33, the injury is very similar to that caused by stem rust. In this case the seed was badly shriveled; hence, the weight of individual kernels was greatly reduced, while the number of kernels per head was not reduced beyond the number normally expected.

The yield of straw was not so greatly reduced by heavy leaf-rust infection as was the yield of other plant parts. This is in agreement with the observations made by Weiss (*19*) to the effect that rust has less effect on the yield of tops than on that of grain. However, it is clearly shown from the experiments here reported that when a heavy infection of leaf rust develops early on a susceptible variety the yield of straw may be reduced by one-third. Furthermore, a greater reduction probably could be expected if soil moisture were low, a factor not introduced into these experiments.

The deterioration and loss in yield of roots because of heavy leaf-rust infection on a susceptible variety suggest that the parasitism of the rust has a very serious effect on the physiology of the host. There seems to be a delicate balance between the health of the top growth and that of the roots. An abundance of rust occurring as late as flowering time is nearly as destructive to the root system as one occurring much earlier. Cereal roots do not store a large quantity of carbohydrates but depend upon a constant translocation from the aerial parts. If carbohydrates were used by the parasitic fungus in the leaves as rapidly as elaborated, a very small quantity would be available for translocation. The results presented here suggest that a sudden reduction in the rate of exchange undoubtedly is reflected in the roots as well as in the tops.

Severe leaf-rust infection in a susceptible variety of wheat may affect certain plant characters that are not obviously associated with yield. In these experiments plants of the susceptible variety which were rusted for long periods required more time to reach heading and maturity than the nonrusted controls. Such plants also were forced to throw out a considerable number of small green tillers late in the season, often after the grain was nearly mature. Whether these tillers would ultimately have produced heads and grain is not known, since it was necessary to remove them in order to terminate the experiments. It is doubtful, however, if this effect would have been seen had the plants not had an abundance of water at all times.

The effect of leaf rust on the water requirement of the susceptible variety was very marked, causing increases of from 31.7 to 104 percent, depending on the length of the rust period. This is at variance with the results reported by Weiss (*19*), who concluded that increases in the water requirement of Marquis wheat because of leaf-rust infection were not significant. It should be pointed out, however, that his leaf-rust infections were low compared with those obtained in these experiments.

Various investigators have reported water requirements for wheat varying from about 250 to nearly 700. In the experiments reported herein, the requirements for the noninfected controls were well within that range, being 544 for Pusa No. 4 and 551.1 for Warden.

The effects of leaf-rust infection on the resistant variety were slight in nearly all cases when compared with those recorded for the susceptible variety. The increases or decreases calculated for Warden were so small in many cases that they are considered of doubtful significance. While there was a tendency for long-continued leaf-rust infection to cause decreases in yield and increases in the growth period and water requirement, the effects were not striking.

SUMMARY

The effect of the intensity and duration of leaf-rust infection on several varieties of wheat was studied with regard to yield, plant characters, and water economy of plants grown under greenhouse conditions.

Three winter-wheat varieties gave unsatisfactory results because of their long growing season, profuse tillering, and tendency to lodge. Two spring-wheat varieties, Pusa No. 4 (susceptible) and Warden (resistant), grown in sealed stone jars, proved to be very satisfactory.

Leaf rust reduced the average yield of the susceptible variety from 42.4 to 93.8 percent, depending upon the length of the infection period.

Reductions in grain yields were due primarily to the production of fewer kernels by rusted plants and secondarily to reduced kernel weight.

The yield of straw was not so greatly reduced by rust infection as was that of grain, but reductions of more than one-third were obtained when the susceptible variety was rusted from the seedling stage to maturity.

Abundant flecking of the leaves of the resistant Warden resulted in a maximum reduction in yield of grain of only 15.2 percent.

Heavy rust infection on the susceptible variety resulted in a rapid and severe deterioration of the roots as indicated by discoloration, a decrease in the number of fibrous roots, and a marked loss in weight.

The number of kernels produced and the height of plant were greatly reduced by prolonged severe rust infection of the susceptible variety. These characters were only slightly affected in the resistant variety.

Plants of the susceptible variety that were heavily rusted for long periods put out many new tillers about the time the grain of the primary tillers began to mature. This also was noted in the resistant variety when flecking was severe.

Leaf-rust infection greatly retarded heading in the susceptible variety and increased the length of the fruiting period, whereas only a slight tendency in this direction was noted for the resistant variety.

Leaf-rust infection increased the water requirement of the susceptible variety 31.7 to 104 percent, based on total dry matter and depending on the length of the rust period. The water requirement of the resistant variety was only very slightly increased by rust infection.

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FUSARIUM-RESISTANT DANISH BALLHEAD CABBAGE[1]

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INTRODUCTION

Wisconsin Hollander cabbage, developed by Jones and Gilman[2] and introduced in 1916,[3] has since that date been used widely as a late storage variety on soils infested with the yellows organism (*Fusarium conglutinans* Wr.). Although in the main it has continued to show a relatively high degree of resistance, there occurs not uncommonly a rather large percentage of mildly diseased plants on heavily infested soils during periods of high temperature. In a recent study Anderson[4] has shown that this variety does not contain the main gene for resistance to yellows described by Walker.[5] Its high tolerance of the parasite is probably due to secondary genes which are responsible for various degrees of resistance at moderate temperatures but not at high temperatures.

Wisconsin Hollander is distinct from the Danish Ballhead variety, from which it was derived, in being later in maturity and in producing characteristically a larger plant with a somewhat flattened head. Because of the need for a highly resistant selection of the true Danish Ballhead type the study reported herein was undertaken. Beginning in 1927, selections were made from Danish Ballhead and continued until the present time. The aim has been to secure lines homozygous for the main gene for resistance which will conform to the acceptable commercial stocks of Danish Ballhead.

EXPERIMENTAL RESULTS

The methods of selection, seed growing, and testing for resistance have already been described.[5]

Seed of the Danish Ballhead variety was sown on yellows-infested soil in a greenhouse bench in the spring of 1927. Out of several hundred seedlings about 25 survived. These were transplanted to the field and in the autumn those most closely resembling ideal Danish Ballhead were saved. Three of these plants (nos. 19, 20, and 27) blossomed in the greenhouse during the ensuing winter, and seed from self-pollination and sib crossing was secured. A fourth seed plant (no. 15) was secured from an individual selected in a planting of the

[1] Received for publication Aug. 10, 1934; issued February 1935. Cooperative investigations of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology, University of Wisconsin.

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Danish Ballhead variety on yellows-infested soil in Racine County, Wis.

The progenies were tested on yellows-infested soil in the field in 1928. All plants showing any sign of yellows during the growing season were placed in the susceptible class. The selfed progenies from plants 19 and 20 and the sib crosses made between those two plants segregated fairly closely in the ratio of 3 resistant to 1 susceptible (table 1), although the numbers are rather small to be significant. No selfed progenies were secured from plants 15 and 27, and insofar as the resistant and susceptible genes are concerned the populations of the hybrid progenies were too small to indicate the constitution of the parent plants.

TABLE 1.—*Segregation into resistant and susceptible classes in progenies from resistant individuals selected from Danish Ballhead variety, field trials 1928*

Progeny no.	Healthy	Diseased	Progeny no.	Healthy	Diseased
	Number	*Number*		*Number*	*Number*
19s [1]	16	5	15×27 [2]	10	11
20s [1]	9	4	27×15 [2]	2	3
19×20 [2]	43	16			
20×19 [2]	33	14	Total	117	63
15×20 [2]	4	10			

[1] Progeny from self-pollination.
[2] Hybrid progeny secured by working a single brush over blossoms of the 2 plants.

The survivors from this test were rogued rigidly and only those resembling the ideal Danish Ballhead type were saved. These plants were grown to blossom in the greenhouse in the winter of 1928–29. The plants were paired and a single brush was worked over the bagged blossoms of each pair. The results of trials of the hybrid progenies on yellows-infested soil in the field in 1929 are given in table 2. Of the 26 progenies, 13 showed no yellows. This indicated that at least one parent of each of the disease-free progenies was homozygous for the resistant gene.

TABLE 2.—*Percentage of yellows in progenies from brother-sister matings between resistant individuals selected in 1928 (table 1), field trials 1929*

Cross or variety	Total plants	Yellows	Cross or variety	Total plants	Yellows	Cross or variety	Total plants	Yellows
	Number	*Percent*		*Number*	*Percent*		*Number*	*Percent*
102×113	33	12	113×135	64	17	129×136	74	0
103×104	27	0	115×110	139	0	132×109	15	0
104×103	42	14	117×112	45	0	133×107	89	0
105×106	30	20	119×121	55	18	134×112	61	0
106×105	40	10	121×113	73	3	135×113	146	10
109×132	33	0	121×119	135	26	136×129	84	0
110×115	90	0	124×125	52	21	Danish Ballhead	293	91
112×134	91	0	125×124	80	26			
113×102	72	0	126×118	58	3			
113×121	50	0	128×127	72	29			

Progenies from reciprocal crosses 129×136 and 136×129 were outstanding for their uniformity, vigor, and proximity in type to Danish Ballhead. Plant 129 had been selected from sib-cross progeny 20×19 and plant 136 from sib-cross progeny 27×15. Thirty-five selected plants from 129×136 and its reciprocal were brought to

blossom in the greenhouse in the winter of 1929–30. Selfed progenies were secured from 30 of these. It was not certain that plants 129 and 136 were both homozygous for resistance, nor, therefore, that any plant in the hybrid progeny was homozygous for resistance. Since the amount of seed secured upon self-pollination is often very small, the reduced population of the selfed progeny sometimes limits the accuracy of the field test. Back crosses were therefore made on each plant with pollen from plants of a homozygous susceptible line. The back-cross progenies were tested in the greenhouse on yellows-infested soil held at 22° to 24° C., so as to give optimum conditions for complete and rapid expression of susceptibility.

In table 3 are given the numbers of healthy and diseased plants in the selfed progenies tested in the field and the behavior of the back cross under controlled conditions in the greenhouse. It will be seen that 21 of the selfed progenies showed no yellows in the field, and that in each case the back cross of the parent plant with a susceptible parent remained perfectly healthy. Nine selfed progenies segregated into resistant and susceptible classes. Most of the selfed progenies in this group segregated fairly closely to a 3 to 1 ratio, and the back-cross progenies were close to a 1 to 1 ratio. Progenies 202 and 203, however, gave a very poor fit, but the back crosses on these parent plants gave a close fit to a 1 to 1 ratio. It is possible that secondary factors may have modified the expression of susceptibility in some of the progenies. The reaction of the back crosses of the 5 plants that produced no seed upon self-pollination indicated that 1 was homozygous for resistance and 4 were heterozygous. The type of segregation that prevailed indicated rather strongly that the same gene for resistance was concerned as has been previously described in other resistant lines derived from other varieties of cabbage.[6]

TABLE 3.—*Segregation of resistant and susceptible plants in progenies from individuals in crosses 129×136 and 136×129 (table 2) when self-pollinated and when back-crossed with pollen from homozygous susceptible plants, 1930*

Genotype	Parent plant no.	Selfed		Back cross		Genotype	Parent plant no.	Selfed		Back cross	
		Healthy	Diseased	Healthy	Diseased			Healthy	Diseased	Healthy	Diseased
	Number	*Number*	*Number*	*Number*	*Number*			*Number*	*Number*	*Number*	*Number*
Homozygous (*RR*)	200	9	0	45	0	Heterozygous (*Rr*)	202	3	9	14	9
	201	101	0	48	0		203	44	1	18	14
	206	10	0	45	0		204	24	4		
	208	17	0	44	0		207			33	12
	210	19	0	64	0		217	13	4	26	16
	211	10	0	45	0		219			14	11
	215	39	0	44	0		222	63	20		
	216	3	0	42	0		223			13	12
	218	20	0	26	0		225	24	3		
	221	4	0	42	0		228	39	8	21	23
	224	31	0	8	0		230			17	28
	227	4	0	45	0		232	106	44	25	18
	229	22	0	45	0		247	120	30	12	20
	231	62	0	34	0						
	234	21	0	25	0	Total	13	436	123	193	163
	235	24	0	30	0						
	236	21	0	25	0						
	241	16	0	26	0						
	242	7	0	30	0						
	243	11	0	25	0						
	244			27	0						
	248	42	0	30	0						
Total	22	493	0	795	0						

[6] WALKER, J. C. See footnote 5.

Five of the completely resistant selfed progenies listed in table 3 were selected as representing the best type in the group. The most desirable plants from these were selected in the autumn of 1930. To avoid loss of vigor which might result from continued self-pollination these plants were placed in a single plantation in 1931 and allowed to cross-pollinate naturally. The seed from all plants was harvested in a single lot and labeled 3331B. This lot of seed was subjected to further tests for resistance and type. In table 4 are given the results of tests for resistance on yellows-infested soil in the field and greenhouse in comparison with a number of commercial lots of Wisconsin Hollander, Danish Ballhead, and Penn State Ballhead.

TABLE 4.—*Comparison of yellows resistant Ballhead (3331B) with Danish Ballhead, Penn State Ballhead, and Wisconsin Hollander on yellows-infested soil in the greenhouse and in the field*

Location of test	Year of test	Strain	Lot no.	Plants tested	Plants diseased
				Number	*Percent*
Greenhouse	1932	Resistant Ballhead	3331B	100	0
		Wisconsin Hollander	8311	65	82
		do	8312	88	67
		Danish Ballhead	10311	93	90
Field	1932	Resistant Ballhead	3331B	250	0
		Wisconsin Hollander	830A	250	37
		do	830B	200	47
		do	830C	200	22
		do	831A	200	42
		do	831B	200	44
		do	8311	200	26
		do	8312	200	18
		Danish Ballhead	10311	200	90
		Penn State Ballhead		150	100
Do	1933	Resistant Ballhead	3331B	223	0
		Wisconsin Hollander	225C	280	21
		do	225H	387	11
		do	830A	263	17
		do	8311	241	43
		do	8321	274	41
		do	8322	174	13
		do	3707	285	16
		Penn State Ballhead		301	99

In the greenhouse, where the average soil temperature was about 22° C., Wisconsin Hollander showed a high percentage of yellows, in accord with previous experience.[7] No. 3331B was completely resistant. In the field the latter strain showed no disease throughout 1932 and 1933. Danish Ballhead and Penn State Ballhead were 90 to 100 percent diseased. The Wisconsin Hollander lots varied from 18 to 47 percent diseased plants in 1932 and from 11 to 43 percent in 1933.

Since the completely resistant progenies passed this high degree of resistance on to the next generation, in which no evidence of segregation occurred either in the field or at high constant soil temperatures, it is evident that the five parent plants were homozygous for resistance. This conclusion is strengthened further by the fact that when those five individuals were crossed with homozygous susceptible plants all F_1 progenies were completely resistant.

Up to this point the development of a homozygous resistant line has been discussed. It has been pointed out that one aim in the inves-

[7] WALKER, J. C., and SMITH, R. EFFECT OF ENVIRONMENTAL FACTORS UPON THE RESISTANCE OF CABBAGE TO YELLOWS. Jour. Agr. Research 41: 1–15, illus. 1930.

tigation was to combine resistance with Ballhead type. Throughout the selection program off-type plants were discarded and only those conforming reasonably closely to the Ballhead type were saved. Since the homozygous resistant seed lot, 3331B, represented the most satisfactory stock secured, it was planted alongside 2 commercial lots of Danish Ballhead and 1 lot of Wisconsin Hollander at Madison, Wis., in 1933, on soil where the infestation by the yellows organism was slight. A comparison was also made the same year at Appleton, Wis., between another commercial lot of Danish Ballhead and 3331B.

The Madison planting was made on June 21. The first harvest of mature heads was made in strain 2 of Danish Ballhead and in strain 3331B on the eighty-ninth day after transplanting. The first harvest in strain 1 of Danish Ballhead was made on the ninety-sixth day; this stage was reached in Wisconsin Hollander on the one hundred and fourth day. Thus the resistant strain was as early in maturity as Danish Ballhead and about 2 weeks earlier than Wisconsin Hollander.

As the plants matured certain physical measurements indicated in table 5 were made at Madison and at Appleton. It is to be seen that in all important features the resistant strain resembled the Danish Ballhead strains rather than Wisconsin Hollander. It exceeded all strains in the proportion of head weight to weight of outer leaves and produced the most nearly spherical heads. The data show that in addition to being later in maturity Wisconsin Hollander consists of taller plants with greater leaf spread and longer stems. It also had on an average flatter heads than all Ballhead strains except strain 2, and the cores were longer in relation to length of head. A representative plant of 3331B and heads of 3331B and Danish Ballhead (strain 1) are shown in figure 1.

DISCUSSION AND SUMMARY

This study has shown that homozygous resistant lines of cabbage of the Danish Ballhead type can be secured by selection from the commercial variety. The most important step in this process is the thorough elimination at the outset of all plants of the susceptible or intermediate resistant types and retention of only those individuals that contain the main gene for resistance. By rigid selection within the progenies from such survivors it was possible to secure a combination of complete resistance with type equivalent to the accepted commercial standard of Danish Ballhead. The resistant strain (3331B) is being increased for commercial use and will be designated hereafter as Wisconsin Ballhead to distinguish it from susceptible strains of the Danish Ballhead variety and from Wisconsin Hollander.

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TABLE 5.—*Physical measurements of Danish Ballhead, resistant Ballhead, and Wisconsin Hollander grown at Madison and Appleton, Wis., 1933*

Variety and strain	Location of trial	Plants measured	Height of plant	Spread of plant	Outer leaves	Weight of outer leaves	Length of stem [1]	Diameter of stem [2]
Danish Ballhead:		*Number*	*Centimeters*	*Centimeters*	*Number*	*Grams*	*Centimeters*	*Centimeters*
Strain 1	Madison	25	37.4±0.60	73.3±0.87	19.2±0.44	1,419.4±45.5	12.9±0.40	5.5±0.09
Strain 2	do	26	36.7± .62	70.6± .33	17.8± .43	1,263.9±40.9	10.6± .31	4.5± .11
Strain 3	Appleton	25	34.3± .41	73.8±1.10	21.1± .73	1,212.2±41.0	12.1± .22	5.1± .09
Resistant Ballhead:								
3331B	Madison	32	33.7± .45	65.3± .91	17.2± .31	1,008.3±24.3	9.4± .19	5.4± .06
Do	Appleton	25	34.3± .50	72.7± .97	18.8± .47	1,084.4±45.4	10.4± .22	5.3± .06
Wisconsin Hollander, 8312	Madison	50	46.4± .46	80.9± .56	17.4± .31	1,631.4±29.5	17.1± .34	5.6± .04

Variety and strain	Weight of head	Ratio of weight of outer leaves to weight of head	Length of head	Diameter of head [3]	Length of core	Diameter of core [4]	Ratio of diameter of head to length of head	Ratio of length of core to length of head
Danish Ballhead:	*Grams*		*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*		
Strain 1	1,897.4±48.9	1.0 : 1.34	15.7±0.16	16.8±0.22	9.9±0.22	3.7±0.07	1.0 : 0.93	1.0 : 1.59
Strain 2	1,903.1±45.2	1.0 : 1.51	15.3± .22	16.9± .23	9.3± .24	3.3± .05	1.0 : .91	1.0 : 1.65
Strain 3	1,660.6±45.6	1.0 : 1.37	14.9± .13	15.6± .22	9.5± .15	3.5± .04	1.0 : .96	1.0 : 1.57
Resistant Ballhead:								
3331B	1,669.8±48.0	1.0 : 1.66	15.6± .17	15.7± .15	10.1± .14	3.6± .03	1.0 : .99	1.0 : 1.54
Do	1,736.2±44.8	1.0 : 1.60	16.0± .21	15.7± .17	9.7± .25	3.4± .05	1.0 : 1.02	1.0 : 1.65
Wisconsin Hollander, 8312	2,255.6±44.3	1.0 : 1.38	16.7± .12	18.4± .16	12.2± .15	3.9± .03	1.0 : .91	1.0 : 1.37

[1] Taken from soil line to base of head.
[2] Taken at base of head.
[3] Taken at point of maximum diameter.
[4] Taken midway between base and apex.

FIGURE 1.—*A*, Typical mature plant of Wisconsin Ballhead (3331B); *B*, head of Danish Ballhead; *C*, head of Wisconsin Ballhead (3331B). The two strains are quite similar in type and season. Wisconsin Ballhead is resistant to yellows while Danish Ballhead is susceptible.

DIFFERENTIAL RESPONSE OF CORN VARIETIES TO FERTILITY LEVELS AND TO SEASONS[1]

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INTRODUCTION

The extent to which results from varietal experiments can be applied safely under growth conditions differing from those of the experiments is an element of considerable uncertainty in agronomic interpretation. The productivity of varietal plots on experiment station fields frequently is very different from that of farms in the same region. Often many soil types and farms of varying degrees of productivity must be served by a single experimental field. If certain varieties are particularly well suited to either the better or the poorer soils, they should be identified and recommended accordingly.

REVIEW OF LITERATURE

The principle of regional adaptation is usually recognized in reports on experiments with corn varieties. In only a few papers, however, have quantitative evaluations of different varietal adaptation to different soils and seasons been reported.

Kiesselbach and Keim (*4*)[3] found pronounced differences in the morphology and rate of development of strains of corn that were adapted to eastern and to western Nebraska.

Mooers (*6, 7, 8*) concluded from a series of experiments in Tennessee that varieties may show quite different productive relationships, depending upon soil fertility and number of plants per unit area. He suggested the location of varietal curves for yield with reference to the yield of a standard variety at different levels of soil fertility. He also suggested that varieties probably respond differently to such climatic limitations as length of day and humidity within the area to which they are adapted.

Mangelsdorf (*5*) found that some varieties were widely adapted to regional conditions in Texas. Others exhibited a medium or narrow range of adaptation, being superior in some regions and inferior in others. Varieties also differed greatly in their response to time of planting.

Hoffer (*3*) reported that selfed lines and their crosses differed widely in the absorption of certain essential mineral nutrients and of iron and aluminum salts.

Goodding and Kiesselbach (*2*) found no important heritable differences in the plant characteristics or grain yield of corn varieties

[1] Received for publication Aug. 22, 1934; issued February 1935. Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Acknowledgment is gratefully made of valuable suggestions and criticisms by F. D. Richey, Chief, Bureau of Plant Industry, U. S. Department of Agriculture, both in planning the experiment and in preparing the manuscript.

[3] Reference is made by number (italic) to Literature Cited, page 1000.

selected by farmers on upland and on bottom land in eastern Nebraska. They point out, however, that these soils were not deficient in phosphorus to the extent that phosphorus fertilizers would materially hasten the maturity of corn, and, further, that rather pronounced hereditary differences might prevail where the comparison is between corn from upland and bottom land if the latter is subject to subirrigation or overflow.

MATERIAL AND METHODS

An experiment bearing on these problems was begun at the Ohio Agricultural Experiment Station in 1928 in cooperation with the Bureau of Plant Industry, United States Department of Agriculture. Four approximate gradations of vertility, designated as "levels", were established by the addition of manure and fertilizer to four adjacent rectangular areas of Canfield silt loam. This soil is derived from sandstone and shale and is naturally low in available nutrients but responds readily to fertilizers and manure. The treatments were as follows:

Level A, no additions.
Level B, 4 tons of manure; 100 pounds of 16-percent superphosphate, broadcast; 100 pounds of 4–12–4 in the hill.
Level C received double the treatment of level B.
Level D received double the treatment of level C.

The hill applications were made by hand with a device which placed the fertilizer in a circular band about 1 inch wide with an inside diameter of 3 inches. In general, the stands were satisfactory on all levels. The field accommodated 100 rows, each 40 hills long, with 10 of the 40 hills on each level. The experiment was conducted on three fields in a corn-oats-wheat rotation; the results with corn only are considered in this paper.

Varieties were grown across the four fertility levels in fourfold or fivefold replication. Hybrids only were used in 1928 and both hybrids and open-pollinated varieties in subsequent years.

GENERAL RESPONSE TO FERTILITY LEVELS

The yield, percentage of dry matter in the ears at harvest, and the number of days from planting to silking are shown in table 1 and figure 1. The values charted are the means for all varieties in 1928 and for six comparable varieties in the succeeding seasons. The mean response to the 4 fertility levels differed greatly in the 5 seasons. The heavy applications of fertilizer and manure on the high levels resulted in lower yields in 1930, 1931, and 1932.

TABLE 1.—*Average acre yield, percentages of dry matter in ears at harvest, and periods from planting to silking*

Year	Acre yield				Dry matter in ears at harvest				Period from planting to silking			
	Level A	Level B	Level C	Level D	Level A	Level B	Level C	Level D	Level A	Level B	Level C	Level D
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Percent*	*Percent*	*Percent*	*Percent*	*Days*	*Days*	*Days*	*Days*
1928	36	42	53	58	50	55	59	61	95	91	87	86
1929	24	33	43	58	56	62	65	63	83	79	78	76
1930	18	27	26	21	52	59	62	61	102	92	87	85
1931	46	56	68	62	62	64	69	66	82	78	74	74
1932	44	66	73	69	61	66	68	70	86	80	77	73

The varieties silked from 7 to 17 days earlier on level D (heavily fertilized) than on level A (not fertilized). The variation in earlier silking was associated with variation in summer rainfall (table 2 and fig. 2).

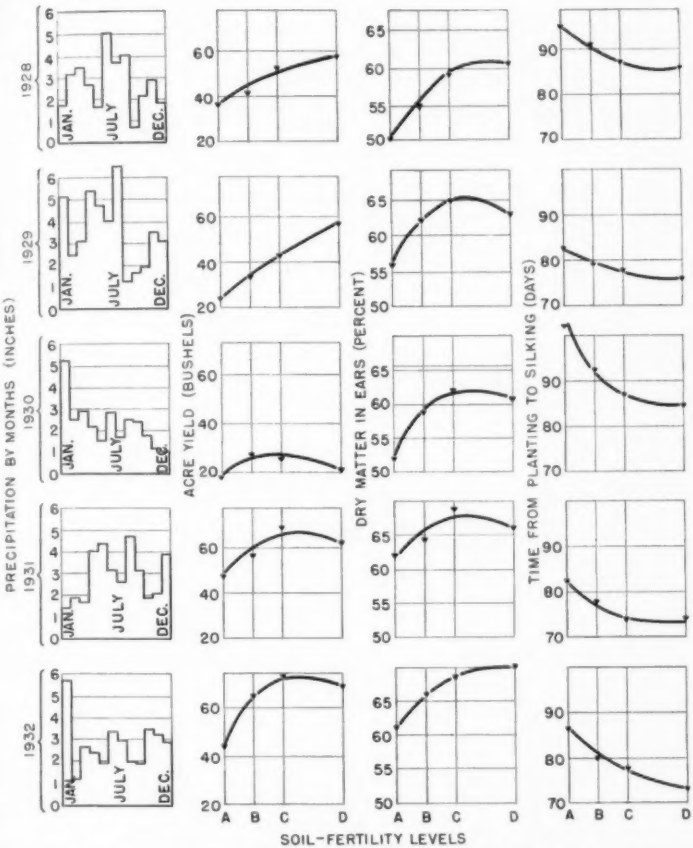

FIGURE 1.—Monthly precipitation and average acre yield, percentage of dry matter in ears at harvest, and periods from planting to silking across the four fertility levels in the different seasons. (Freehand curves.)

The plants on the D level silked earlier than those on the C level in 1928, 1929, 1930, and 1932 and on the same date in 1931. The plants on D, therefore, had developed more rapidly than those on C in the earlier part of seasons 1928, 1929, 1930, and 1932 and at about the same rate in 1931. The percentage of dry matter in the ears at

harvest, however, was lower on the D than on the C level in 3 of the 5 seasons. Percentage of dry matter in the ears on standing plants at harvest may be taken as a measure of the rate of development for the season as a whole. In 3 of the 5 seasons the plants on level C had developed more rapidly, but in 2 of these seasons, development during the presilking stage was more rapid on level D, as measured by silking date. There was, accordingly, a reversal of the influence of fertility on the rate of development from the presilking to the post-silking stages during these two seasons. This suggests a danger in using silking date as a measure of rate of development for the season as a whole in fertility experiments.

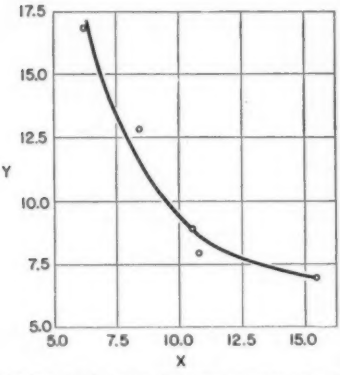

FIGURE 2.—Relation between summer rainfall and difference in rate of plant development between low and high fertility levels. y=number of days difference in silking dates between level A (not fertilized) and level D (heavily fertilized); x=total rainfall in inches during May, June, and July. (Freehand curves.)

A detailed attempt to correlate these various seasonal responses with the peculiarities of weather in each year would be largely speculative and beyond the intent of this paper. It seems well demonstrated, however, that season may markedly affect the plant response to the different levels.

TABLE 2.—*Rainfall in presilking period and difference in time from planting to silking between level A (not fertilized) and level D (heavily fertilized)*

Year	Rainfall		Difference between levels in time from planting to silking
	July	May, June, July	
	Inches	*Inches*	*Days*
1928	3.75	10.61	9
1929	6.79	15.73	7
1930	1.71	6.16	17
1931	2.97	10.91	8
1932	3.14	8.51	13

VARIETAL RESPONSE TO FERTILITY LEVELS AND TO SEASONS

The varietal response to fertility levels and to seasons was studied (1) by analysis of the variance, (2) by graphs of actual yields and of regression, and (3) by comparisons of the degree of response among different varieties in different fertility levels based on deviations from the varietal means within the different seasons.

ANALYSIS OF VARIANCE

By the use of Fisher's method (*1*), variance was analyzed for 6 comparable varieties grown on each of the 4 fertility levels during the 4 seasons 1929 to 1932. The average yield of a single variety on a single level in a single season was the unit in the computations.

The data are shown in table 3. The interaction between fertility level and season shows that the yields on the four levels bore decidedly different relations to each other in the different seasons. The use of a 3-field rotation complicated the interpretation of this point.

TABLE 3.—*Analysis of variance of 6 comparable varieties on 4 fertility levels in 4 seasons*

Variance due to—	Degree of freedom	Sum of squared deviations	Mean square	Standard deviation	Odds that mean square values are not random
Variety	5	989.80	197.96		>99:1
Fertility level	3	5,517.37	1,839.12		>99:1
Season	3	24,618.78	8,206.26		>99:1
Interaction of variety with fertility level	15	148.82	9.92		<19:1
Interaction of variety with season	15	1,127.91	75.19		>99:1
Interaction of fertility level with season	9	1,779.59	197.73		>99:1
Error	45	503.97	11.20	3.35	
Total	95	34,686.24			

The significance of varietal differences is clear. The interaction between variety and fertility level, however, is nonsignificant for the period as a whole (odds<19 to 1). It will be shown later that a significant difference in the response of varieties to fertility levels did occur in two of the seasons.

The interaction of variety with season is highly significant (odds>99 to 1). This indicates that the relative standing of varieties was significantly different in different seasons. It does not follow that the influence of season on varietal standing differed in kind from the influence of fertility level, since the variation in average yields resulting from seasonal variation was much greater than that resulting from differences in fertility level. It happened, however, that the two seasons 1931 and 1932 differed in average yields by only a few bushels per acre. When the square root of Fisher's *A* value (*1, sec. 40*) was used, the variability assignable to individual varietal units due to season in these 2 years was found to be only 2.39 bushels per acre, while that due to fertility level was 11.01. In spite of the much lower variability due to season, the interaction of variety with season was highly significant (odds>99 to 1), but there was no significant interaction of variety with fertility level. These data show that the major part of the differential varietal performance may be attributed to climatic rather than to soil differences.

It should be pointed out that the differential varietal response between seasons could have been influenced by differences in seed condition or in the sampling of varieties from year to year. Seed condition was good throughout, however, and seed representing many plants was used. This possible source of error is accordingly not thought to have been important.

GRAPHIC STUDY OF VARIETAL RESPONSE TO FERTILITY LEVELS AND TO SEASONS

Varieties that were grown in at least 3 of the years may be grouped as follows:

Ohio open-pollinated sorts:

No. 1, Medina Pride, and no. 2, Clarage, both locally adapted in northeastern Ohio.

No. 3, Leaming, adapted to central Ohio.

Iowa hybrids of inbred lines:
Nos. 4, 5, and 6. (No. 6 was grown only in the last three seasons.)
Connecticut hybrid of inbred lines:
No. 7.

The approximate ranking in time of maturity was: No. 6, earliest; nos. 1, 2, 3, 4, 5, 7, latest.

The yields of comparable varieties on the different levels are shown in figure 3. The data for 1928 are not included because the variety list was entirely changed after that year. Only nos. 2, 3, 5, 6, and 7 are shown in the figure, to avoid an undue complication of lines. No. 3, the strain of Leaming, seems relatively better at the low levels than at the high in 3 of the years.

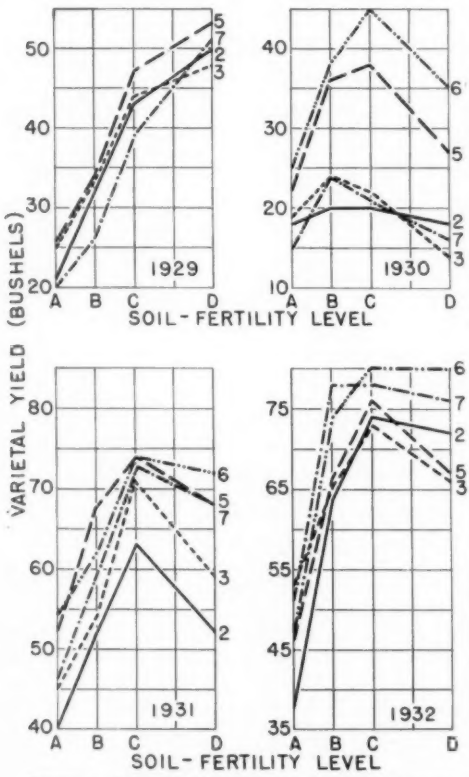

FIGURE 3.—Relative yield performance of 5 varieties across the 4 fertility levels in different seasons.

The hybrids, as a whole, were much more productive than the open-pollinated varieties. The Iowa hybrids, nos. 4, 5, and 6, however, responded very differently from the Connecticut hybrid, no. 7. The Iowa hybrids tended to be relatively productive at all levels in all seasons, whereas the Connecticut hybrid took its place with the open-pollinated sorts in the drought years, 1929 and 1930, and with the Iowa hybrids in the more humid years. Considering the less humid conditions under which the Iowa material was selected, this tendency might have been expected. The difference between the upper and lower lines for 1930 in figure 3 may represent the difference in the way a cornfield would yield in a dry season, depending upon whether varieties adapted to less or to more humid conditions were being grown.

The fertility treatment on level C in 1930 was excessive for the Ohio and Connecticut sorts. The yields of the western hybrids, however, were largest on this level. This may be interpreted as a differential tolerance to the effect of fertilizers and manure in a dry season.

The Iowa hybrid designated as no. 6 had the most satisfactory yield record in the experiment. In addition to being the earliest in maturity in 12 comparisons, it was first in yield 9 times, second twice, and third once. This demonstrates the fact that some varieties may yield very well under a wide range of conditions.

The regression of individual variety yields on the yields of the 4 fertility levels for the 6 varieties that were grown throughout the 4-year period is shown in figure 4. The averages for 4 seasons are used, and the vertical and horizontal scales are equal so that the means of the 6 varieties at the different levels fall on a straight line with a regression of 1. This weights the levels differently from a weighing based on the treatments they received. It also changes their rank. Variety no. 3, the Leaming strain, is outstanding in its weak response to more favorable growth conditions. The regression of no. 7, the Connecticut hybrid, is almost identical in slope with those of nos. 4 and 5, the Iowa hybrids.

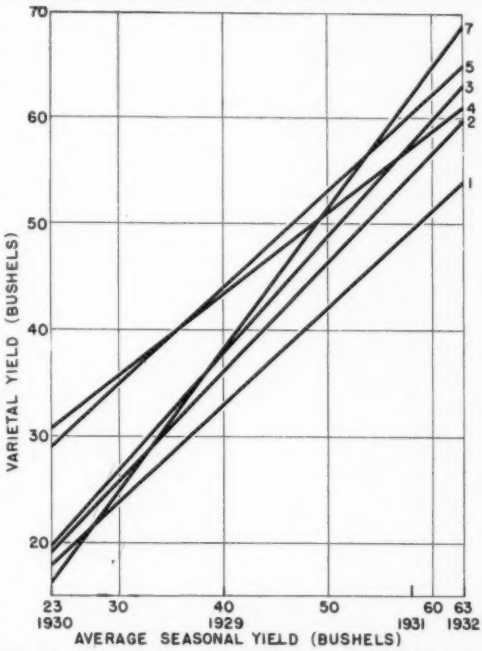

FIGURE 4.—Regressions of 6 comparable varieties across 4 fertility levels with the horizontal scale so adjusted that the 6-variety means fall on a straight line with a regression of 1. (Average of four seasons.)

A very different relation is observed in figure 5, where the regressions of yield of the same 6 varieties are charted across the 4 seasons. The averages for all levels are used, and the vertical and horizontal scales are equal so that the means of the 6 varieties in the different seasons fall on a straight line with a regression of 1. The superiority of nos. 4 and 5, Iowa hybrids, decreases as seasons are more favorable. Conversely, no. 7, the Connecticut hybrid, is obviously a "good season" variety. The principal factors affecting favorableness of season evidently were the amount and the distribution of rainfall.

DEGREE OF RESPONSE AMONG VARIETIES BASED UPON DEVIATIONS FROM VARIETAL MEANS

If varieties respond differently in yield as additions of fertilizers and manure are supplied, the sums of the total deviations of the yields on the different levels from their means should give a measure of the response of the different varieties. Example: If the yields of a variety on levels A, B, and C in a given year are 30, 40, and 50 bushels,

respectively, their mean yield is 40. Deviations from this mean are −10, 0, and 10, the sum of which, disregarding signs, gives a total deviation of 20. The greater the additional yield of a variety per unit of manure and fertilizer applied, the larger its response value will be. Using only levels A, B, and C, to eliminate in part the complication of reduced yields on level D, where fertilizer application was extremely heavy, such response values for the different varieties were computed. The frequency distribution of these for the 5 years is shown in table 4.

TABLE 4.—*Distribution of values representing varietal response to additions of fertilizers and manure*

Year	Response value											Odds against purely random distribution
	5–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	
1928				3	3	3	7	3	1			>19:1
1929				1	2	3	4	7	1	1	1	>99:1
1930	2	2	1	3	2							>99:1
1931			2	3	5	1						<19:1
1932	3	6	2									<19:1

The statistical significance of the departure of these distributions from random is well established for 2 of the 5 years. Clearly, a differential varietal response to fertilizers obtains in some seasons.

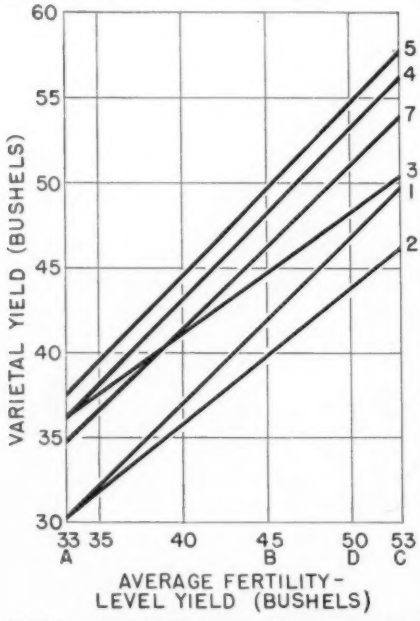

FIGURE 5.—Regressions of 6 comparable varieties across 4 seasons with the horizontal scale so adjusted that the 6-variety means fall on a straight line with regression of 1. (Average of four fertility levels.)

RELATION OF RESPONSE TO ABSOLUTE VARIETAL YIELD

Varietal response, as described above, was related to varietal yield in 3 of the 5 seasons. Regressions indicated that, as yield among varieties increased 1 bushel per acre, response increased respectively 0.34, 0.50, and 1.07 bushels in 1928, 1929, and 1930; or, as yield increased 1 percent of its mean, response increased 0.5, 0.5, and 1.6 percent of its mean. Compare, for example, two varieties, A, high yielding, and B, low yielding. Variety A would be expected to have given the larger response to fertilizers and manure in 1928 and 1929. But on a percentage basis the expected difference in response would be only about half as great as the percentage difference in yield. In the drought year 1930, however, the expected percentage difference in response would be 1.6 times greater than the percentage difference

in yield. Response showed no measurable relation to yield in either 1931 or 1932.

Relation of Response in Yield to Response in Rate of Development

Response in percentage of dry matter in the ears at harvest was computed by the same method as that described above for response in yield; that is, the total deviation from the mean across levels A, B, and C was found. The coefficients of correlation for this measure with that for the response in yield are shown in table 5.

Table 5.—*Coefficients of correlation between the response values for dry matter and those for yield*

Year	r	Standard deviation	Year	r	Standard deviation
1928	0.83	0.070	1931	0.47	0.175
1929	.18	.216	1932	.42	.248
1930	.73	.148			

Since it was obvious from the data that both yield and rate of development were increased and never lessened from level A to level C, the interpretation of these correlations is that those varieties whose responses to manure and fertilizers were relatively large tended somewhat also to be hastened in rate of plant development relatively more by the same treatment.

SUMMARY AND CONCLUSIONS

Hybrid and open-pollinated varieties of corn were grown in five seasons across four "levels" of soil fertility established by additions of fertilizer and manure.

In general, yields were higher and rate of plant development more rapid at the higher levels.

In some seasons, plants under very heavy applications of fertilizer and manure developed rapidly in the presilk stage and slowly in the postsilk stage as compared with plants under more moderate applications.

The difference in rate of plant development between unfertilized and heavily fertilized levels was markedly and progressively greater as rainfall was less.

Differential varietal response in yield among the different levels was clearly significant in two of the seasons but not in the experiment as a whole.

The differential yield response of varieties due to different seasons was, however, consistent and highly significant and was much greater than that due to fertility levels. This seasonal differential response held even between two seasons in which average yields were nearly alike. It seems probable that a change in climatic factors will affect relative varietal performance considerably more than will a quantitative change in available nutrients.

The data corroborate previous reports that a variety may be relatively better at low than at high fertility levels, or vice versa. The differences found, however, seemed to be largely associated with adaptation to different geographical regions.

Certain Iowa hybrid varieties were greatly superior in the drier seasons, but their superiority was progressively less as seasons were more favorable. On the other hand, a Connecticut hybrid was definitely a "good-season" variety. It was shown that a variety may be productive under a wide range of soil and climatic conditions.

Iowa varieties were more tolerant than eastern varieties of the stimulating effect of manurial treatment in the drought year of 1930.

The following relations held with more or less consistency: Varieties with high potential yielding capacity tended to respond more favorably to the higher fertility levels. Relatively strong plant response in yield to the higher levels was associated with relatively strong plant response in rate of development to the higher levels.

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THE RELATION OF THE SUGAR CONTENT AND ODOR OF CLARIFIED EXTRACTS OF PLANTS TO THEIR SUSCEPTIBILITY TO ATTACK BY THE JAPANESE BEETLE [1]

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INTRODUCTION

The investigation upon which this paper is based was undertaken at the Japanese beetle laboratory at Moorestown, N. J., during the summer of 1931, in order to determine the difference, if any, between the sugar content of plants that are immune to attack by the Japanese beetle (*Popillia japonica* Newm.) and those that are favored food plants of this insect. Prior to 1931 considerable work had been done by Metzger and Grant [4] in extracting certain constituents from various plants that are immune to beetle attack. The purpose of this earlier project was to discover substances in these plants that might be repellent to the beetle. It was realized, however, that the immunity might be caused by the absence of an attractant rather than by the presence of a positive repellent. The results obtained by testing extracts of these immune plants against the beetle appeared to confirm the view that they possessed no repellent constituents. If this were true, it seemed probable that favored food plants should possess some chemical characteristic which induces feeding by the beetle. A determination of the sugar content of the attractive plant material appeared to be a logical field for investigation in this connection.

MATERIAL AND METHODS

SELECTION OF PLANT MATERIAL

The plants used in this work were selected as being typical of the various groups represented. Many additional species might have been examined profitably, but in view of the limited time available for this work, inclusion of a larger group was not considered advisable. It was impossible to collect all the material at the same time, but each sample was taken from a plant before its full seasonal development had been reached. It is possible that the time of collection may have influenced the sugar content of certain samples.

ANALYTICAL PROCEDURE [5]

The procedure for each plant was as follows: 78 g of the fresh plant material under consideration was weighed out and cut into small pieces. One gram of pure calcium carbonate was placed in 300 cc of 95-percent ethyl alcohol. The alcohol was heated to the boiling point and the plant material added gradually. The mass

[1] Received for publication Aug. 22, 1934; issued February 1935.
[2] Resigned Aug. 20, 1932.
[3] Resigned Aug. 29, 1933.
[4] METZGER, F. W., and GRANT, D. H. REPELLENCY TO THE JAPANESE BEETLE OF EXTRACTS MADE FROM PLANTS IMMUNE TO ATTACK. U. S. Dept. Agr. Tech. Bull. 299, 22 pp., illus. 1932.
[5] Insofar as possible the procedure followed the methods described in the following publication: ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Ed. 3, 593 pp., illus. Washington, D. C. 1930.

was then boiled for 30 minutes under a reflux condenser. When partially cool the preparation was put in a flask which was then closed with a rubber stopper and stored for future extraction.

For the extraction the liquid was decanted from the container and approximately one-third was placed in each of three 500-cc flasks. The leaves were then divided among three large Soxhlet extractors. Approximately 150 cc of 95-percent alcohol was added to each, and the extraction was continued on a boiling water bath for 24 hours, or until the alcoholic extract gave a negative test with alphanaphthol. All the extracts were then combined and evaporated to a small volume on a water bath. The residual solution, made up to a volume of 100 cc with water, was filtered through a folded filter and washed with 30 cc of water. It was then diluted with water to exactly 150 cc.

Ten cubic centimeters of this solution was treated with neutral lead acetate and alumina cream to determine the quantity of these reagents required in the clarification. The necessary quantity of lead acetate was then added to 100 cc of the extract in a 200-cc volumetric flask. To this was added 2 cc of alumina cream, and the whole solution was shaken. It was then diluted to 200 cc with water, shaken, and filtered, the first 25 cc of filtrate being rejected. The filtrate was next treated with anhydrous sodium carbonate to complete the precipitation of the lead. Following this, the solution was again filtered.

The reducing sugars in the filtrate were determined by putting 25 cc each of Fehling's solutions A and B in a 400-cc beaker and adding 50 cc of the clarified extract. The beaker was immediately placed on an asbestos mat over a burner so regulated that the boiling began in exactly 4 minutes. The boiling was continued for 2 minutes and the solution was quickly filtered through an asbestos mat in a Gooch crucible and the filtrate discarded. The precipitate of copper oxide was then washed thoroughly with water at 60° C., and this washing was also discarded. The crucible was then attached to a clean suction flask. Five cubic centimeters of warm dilute nitric acid was added to dissolve the precipitate, and the crucible was covered with a watch glass. After the precipitate had dissolved, suction was applied and the nitric acid solution was drawn into the flask. The watch glass was then washed with 50 cc of water which was allowed to run into the crucible and which in turn was drawn into the flask. The contents of the flask were then boiled to remove the excess nitric acid. Five cubic centimeters of bromine water were added and the excess bromine boiled off. A slight excess of strong ammonium hydroxide was then added, and the excess of ammonia was boiled off. A slight excess, 3 to 4 cc of 80-percent acetic acid was added, and the solution was then boiled for 1 minute.

After the solution had cooled to room temperature, 10 cc of 30-percent potassium iodide solution was added and the liberated iodine titrated at once with sodium thiosulphate solution which had been standardized against copper. The results are reported in milligrams of reducing sugar as dextrose per gram of collected plant material.

An attempt was made to determine the quantity of sucrose present in the extracts. The analytical method for sucrose, however, depends upon the measurement of optical rotation. In nearly every case the clarified extracts were more or less colored, so the results obtained were unsatisfactory and are not reported. In all the extracts so tested the sucrose content appeared to be small.

DETERMINATION OF BEETLE INJURY

Each plant examined was classified, according to the degree of injury normally sustained because of beetle attack, in one of the following groups:

0=not attacked.
1=lightly attacked.
2=moderately attacked.
3=severely attacked.

The plants were assigned to the various groups after careful consideration by several members of the laboratory staff who had been associated with the project for several years. In each case the consensus of opinion determined the group in which the plants were placed.

DETERMINATION OF ODOR OF CLARIFIED EXTRACTS

In the course of the alcohol extraction of plant material all odoriferous substances are extracted along with the sugars and, for the most part, remain in the extract. The odor of the clarified extracts was noted in every case. In order to minimize the personal element as much as possible, several individuals were called upon to make this determination. In table 1 the odor of the clarified extract follows as closely as possible the classification of Zwaardemaker.[6] Extracts with a fruity odor are, however, so designated instead of being classed as "ethereal" as was done by Zwaardemaker.

EXPERIMENTAL RESULTS

Table 1 contains the results of the determination of the sugar content of the plants considered, together with the odor of the clarified extracts and an estimate of the susceptibility of each plant to beetle attack. The grouping is made in descending order of sugar content. Unless otherwise indicated, the leaves were the portion of the plant upon which the determinations were made.

TABLE 1.—*Sugar content, odor of clarified extract, and susceptibility to beetle attack of the plants under consideration*

Order of sugar content	Name of plant	Susceptibility to attack	Odor	Reducing sugar as dextrose per gram of plant material
				Milligrams
1	Peach, *Amygdalus persica* L., var. Belle	3	Fruit	30.0
2	Peach (fruit), *Amygdalus persica*, var. Early Rose	3	do	29.7
3	English elm, *Ulmus campestris* L	3	do	29.5
4	Apple (fruit), *Malus sylvestris* Mill., var. Williams	3	do	29.4
5	Peach, *Amygdalus persica* L., var. Carman	3	do	29.1
6	Flowering dogwood, *Cornus florida* L	0	Weak fruit	28.8
7	Common petunia (flowers), *Petunia hybrida* Vilm	0	Empyreumatic	27.7
8	Rose, *Rosa* sp., var. pink climber	3	Fruit	27.6
9	Peach, *Amygdalus persica* L., var. Iron Mountain	3	do	26.6
10	Common castor-bean, *Ricinus communis* L	1	do	26.4
11	Northern bayberry, *Myrica carolinensis* Mill	2	do	26.4
12	Common witch-hazel, *Hamamelis virginiana* L	1	Aromatic	26.0
13	London planetree, *Platanus acerifolia* Cy	2	Fruit	25.3
14	Norway maple, *Acer platanoides* L	1	do	24.9
15	Babylon weeping willow, *Salix babylonica* L	2	None	24.7
16	Common persimmon, *Diospyros virginiana* L	1	Fruit	22.8
17	American beech, *Fagus grandifolia* Ehrh	1	do	22.2
18	Bracken, *Pteridium aquilinum* L	2	Fragrant	21.6
19	Turnip, *Brassica rapa* L	0	Allyl	21.2
20	St. Johnswort, *Hypericum perforatum* L	2	Aromatic	21.2
21	Fringed iris, *Iris japonica* Thunb	0	Nonfruit	21.0

[6] ZWAARDEMAKER, H. ODORIFEROUS MATERIALS. In National Research Council, International Critical Tables of Numerical Data, Physics, Chemistry, and Technology. v. 1, pp. 358–361. New York and London. 1926.

TABLE 1.—*Sugar content, odor of clarified extract, and susceptibility to beetle attack of the plants under consideration*—Continued

Order of sugar content	Name of plant	Susceptibility to attack	Odor	Reducing sugar as dextrose per gram of plant material
				Milligrams
22	Apple, *Malus sylvestris* Mill., var. Williams	3	Fruit	20.6
23	Tuliptree, *Liriodendron tulipifera* L.	0	Weak fruit	20.1
24	Apple, *Malus sylvestris* Mill., var. Wealthy	3	Fruit	19.4
25	Coralberry, *Symphoricarpos vulgaris* Michx	0	Fragrant	19.1
26	Ailanthus, *Ailanthus altissima* (Mill.) Swingle	0	do	19.1
27	Bouncing-bet, *Saponaria officinalis* L.	0	Fruit	18.5
28	Pin oak, *Quercus palustris* Muench	2	do	18.1
29	Common sassafras, *Sassafras variifolium* Kuntze	3	do	18.1
30	Apple, *Malus sylvestris* Mill., var. Yellow Transparent	3	do	18.1
31	Common nasturtium, *Tropaeolum majus* L.	0	Fragrant	17.5
32	Tupelo, *Nyssa sylvatica*, Marsh	1	Green-leaf	17.4
33	Common plum, *Prunus domestica* L.	3	Fruit	17.1
34	European grape, *Vitis vinifera* L.	3	Fruit	16.8
35	Common greenbrier, *Smilax rotundifolia* L.	0	Fragrant	16.1
36	Arrowwood, *Viburnum dentatum* L.	2	Fruit	16.1
37	Sweetflag, *Acorus calamus* L.	0	None	15.9
38	Sweetbay, *Magnolia glauca* L.	0	Nonfruit	15.1
39	Smartweed, *Polygonum pennsylvanicum* L.	3	Fruit	14.7
40	Cherry (sour), *Prunus cerasus* L.	3	do	14.7
41	Orange-eye butterflybush, *Buddleia davidi* Franch.	2	Nonfruit	14.7
42	Early deutzia, *Deutzia grandiflora* Sieb. and Zucc.	1	Fragrant	14.6
43	Common ironweed, *Vernonia noveboracensis* (L.) Willd.	0	Fruit	14.6
44	American arborvitae, *Thuja occidentalis* L.	0	Greenleaf	13.1
45	Polygonum, *Polygonum* sp.	3	Fruit	13.0
46	American holly, *Ilex opaca* Ait.	0	Fragrant	12.7
47	Weeping forsythia, *Forsythia suspensa* Vahl	0	Fruit	12.3
48	Japanese honeysuckle, *Lonicera japonica* Thunb.	0	do	12.2
49	Sweet autumn clematis, *Clematis paniculata* Thunb.	0	do	12.2
50	Common pear, *Pyrus communis* L., var. Kieffer	0	None	12.1
51	Gray birch, *Betula populifolia* Marsh	2	Fruit	11.6
52	Threelobe spirea, *Spiraea trilobata* L.	0	Empyreumatic	10.7
53	Hickory, *Hicoria* sp.	0	Fragrant	10.7
54	Scrub pine, *Pinus virginiana* Mill.	1	None	10.6
55	Soybean, *Soja max* Piper	3	Fruit	10.5
56	Common rhubarb, *Rheum rhaponticum* L.	3	Fragrant	9.7
57	Cardinalflower, *Lobelia cardinalis* L.	0	Fruit	9.3
58	Buckthorn plantain, *Plantago lanceolata* L.	0	Fragrant	8.3
59	Rose, *Rosa* sp., var. scarlet climber	0	None	8.1
60	Common milkweed, *Asclepias syriaca* L.	1	do	7.8
61	Indian corn (silk) *Zea mays* L., var. Evergreen	3	Weak fruit	7.8
62	Common lizardtail, *Saururus cernuus* L.	0	Fragrant	7.7
63	Southern red oak, *Quercus falcata* Michx.	0	do	7.7
64	Red cedar, *Juniperus virginiana* L.	0	do	7.4
65	Common meadowbeauty, *Rhexia virginica* L.	2	Aromatic	7.3
66	Pickerelweed, *Pontederia cordata* L.	2	do	6.8
67	Blueflag iris, *Iris versicolor*	0	None	6.5
68	Pink weigela, *Weigela rosea* Lindl.	1	Fruit	6.1
69	Tomato, *Lycopersicon esculentum* Mill.	0	None	6.1
70	Red-leaved Japanese barberry, *Berberis thunbergi* DC., var. *atropurpurea*.	2	Fragrant	5.4
71	Common pearlbush, *Exochorda grandiflora* Lindl.	1	Nonfruit	5.2
72	Compassplant, *Silphium laciniatum* L.	0	None	4.8
73	Chinese beautyberry, *Callicarpa purpurea* Juss.	0	Fruit	4.7
74	Pignut, *Hicoria glabra* (Mill.) Britton	1	Fragrant	4.2
75	Indian corn, *Zea mays* L., var. Evergreen	3	None	4.2
76	Chinese redbud, *Cercis chinensis* Bunge	0	Fragrant	4.1
77	Winter honeysuckle, *Lonicera fragrantissima* Lindl.	0	do	3.9
78	Common lilac, *Syringa vulgaris* L.	0	None	3.7
79	Common petunia, *Petunia hybrida* Vilm.	0	do	3.4
80	Butterfly violet, *Viola papilionacea* Pursh	0	Violet	3.3
81	Lombardy poplar, *Populus nigra* var. *italica* Du Roi	0	Fragrant	2.9
82	White ash, *Fraxinus americana* L.	0	Fruit	2.9
83	Common cockscomb, *Celosia cristata* L.	0	Fragrant	2.4
84	Common four-o'clock, *Mirabilis jalapa* L.	2	do	2.2
85	French marigold (flowers) *Tagetes patula* L.	2	Fruit	1.9
86	Velvet grass, *Notholcus lanatus* L.	0	None	1.7
87	Sunflower heliopsis, *Heliopsis helianthoides* L.	0	Aromatic	1.6
88	Early goldenrod, *Solidago juncea* Ait.	0	Fragrant	1.4
89	Lambsquarters, *Chenopodium album* L.	0	None	1.3
90	Goldenclub, *Orontium aquaticum* L.	0	do	1.0
91	Pricklypear, *Opuntia vulgaris* Mill.	0	Allyl	1.0
92	Sweet mockorange, *Philadelphus coronarius* L.	0	Fragrant	.9
93	Panicle hydrangea, *Hydrangea paniculata* Sieb.	0	None	.8
94	Spearmint, *Mentha spicata* L.	0	Mint	.8
95	European burningbush, *Euonymus europaeus* L.	0	None	.8
96	Common zinnia, *Zinnia elegans* Jacq.	2	do	.4
97	Colorado spruce, *Picea pungens* Engelm.	0	Empyreumatic	.4

Many of the significant data given in table 1 can be considered more readily after they have been condensed. In table 2 the extracts have been arranged according to infestation in sugar-content groups of 5-mg intervals. Table 2 shows that 80.7 percent of the plants in the

TABLE 2.—*Plants in each sugar-content group arranged according to infestation*

Sugar content per gram of plant material (milligrams)	Total plants in group	Immune	Attacked			Total attacked
			Lightly	Moderately	Severely	
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1–5	26	80.7	3.9	11.5	3.9	19.3
5–10	16	50.0	18.7	18.7	12.5	50.0
10–15	17	52.9	11.8	11.8	23.5	47.1
15–20	15	46.7	6.6	13.3	33.3	53.3
20–25	10	30.0	30.0	30.0	10.0	70.0
25–30	13	15.4	15.4	15.4	53.8	84.6

group with the lowest reducing-sugar content are immune to beetle attack, whereas only 15.4 percent are immune in the group with the highest reducing-sugar content. On the other hand, only 1 plant out of 26 in the 0- to 5-mg group, or 3.9 percent, is severely attacked, while in the 25- to 30-mg group 53.8 percent are so attacked.

The extreme right-hand column of table 2 shows significant data when the immune plants are considered in comparison with all the plants which are attacked. In the 0- to 5-mg group the number of plants attacked is 19.3 percent of the group, whereas in the 25- to 30-mg group it is 84.6 percent.

A further significant trend is shown by the data in table 3, where the plants in each infestation group are arranged according to sugar content. Forty-two percent of the plants immune to attack occur in the group containing 0 to 5 mg of reducing sugar; only 4 percent fall within the group containing 25 to 30 mg. Five percent of the plants severely attacked are among those with the lowest sugar content, while 35 percent are in the group with the highest sugar content. These data indicate a significant trend toward immunity to beetle attack in plants with a low sugar content and toward severe attack in plants with a high sugar content. When the odor of the clarified extracts is considered in addition to their sugar content this trend is much more definite.

TABLE 3.—*Plants in each infestation group arranged according to sugar content*

Susceptibility to attack	Plants	Plants in each sugar-content group of—					
		0–5 mg	5–10 mg	10–15 mg	15–20 mg	20–25 mg	25–30 mg
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Immune	50	42.0	16.0	18.0	14.0	6.0	4.0
Light	12	8.3	25.0	16.7	8.3	25.0	16.7
Moderate	15	20.0	20.0	13.3	13.3	20.0	13.3
Severe	20	5.0	10.0	20.0	25.0	5.0	35.0

As indicated in table 2, there are 13 species of plants with a sugar content of 25 to 30 mg. Eighty-four and six-tenths percent of this number are attacked. The clarified extracts of 9 of the plants, or

69.2 percent, are characterized by a fruitlike odor. Of the 2 plants in this group not attacked by the beetle, one, the flower of common petunia, does not have a fruity odor, and the other, flowering dogwood, is usually found in shady locations where beetles do not congregate in large numbers.

In the lowest sugar-content group only 19.3 percent of the plants are attacked, and the only one with a fruitlike odor is French mari-

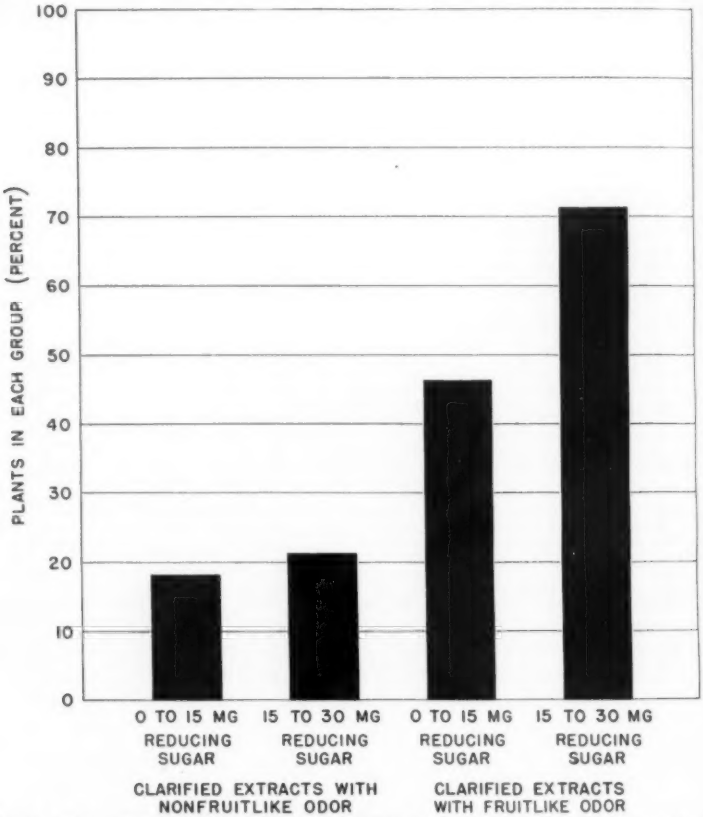

FIGURE 1.—Percentages of moderately and heavily attacked plants in groups separated according to odor and sugar content.

gold, the flowers being the parts injured by the beetle. The single plant in this division that is indicated as severely attacked is corn, the leaves being the part analyzed. It is believed that the feeding on corn leaves is induced, to a considerable degree, by the movement of the beetles to them from the silk after that portion of the plant is consumed. Nineteen of the twenty-one immune plants have a clarified extract without a fruity odor, the exceptions being white ash and Chinese beautyberry. Another important factor to be considered is that the extracts of eight of the immune plants of this range had no appreciable odor of any kind.

Economic injury does not usually occur on plants here designated as lightly attacked, and in table 4 these are grouped with the immune plants. The data given in table 4 are a summary of the foregoing where the plants have been grouped in two sugar-content groups according to the odor of the clarified extracts and according to their susceptibility to beetle attack. Separating the plants into groups, first on the basis of odor, and then subdividing these again into two groups according to the sugar content, the percentage of plants falling in each subgroup is shown in figure 1.

TABLE 4.—*Plants grouped according to the odor of the clarified extract and degree of infestation in two sugar-content divisions*

Sugar content of plants (mg)	Clarified extract without a fruity odor			Clarified extract with a fruity odor		
	Plants	Infestation		Plants	Infestation	
		Absent or light	Moderate to heavy		Absent or light	Moderate to heavy
	Number	*Percent*	*Percent*	*Number*	*Percent*	*Percent*
0 to 15	44	81.8	18.2	15	53.3	46.7
15 to 30	14	78.5	21.5	24	29.1	70.9

A study of table 4 and figure 1 shows very clearly the preference of the beetles for plants whose clarified extracts possess a fruitlike odor and for those having a high sugar content.

There has been considerable conjecture as to whether certain plants growing in areas as yet uninfested by the Japanese beetle will be severely injured when the insect becomes abundant in those areas. From the results presented in this paper it appears unlikely that a plant will be severely attacked by the beetle if the reducing-sugar content is less than 15 mg per gram of plant material and the clarified extract is not characterized by a fruitlike odor. If, on the other hand, the plant has a sugar content of 15 or more milligrams of reducing sugar as dextrose per gram of material and the clarified extract possesses a fruity odor it is probable that it will become a favored food of the beetle.

The foregoing indications, however, must not be strictly applied to plants growing in the shade, or to those in which the epidermis of the leaves is of such a nature that the insect cannot readily feed upon it. This would exclude most evergreens and plants with hard, waxy, or pubescent leaves such as sweetbay, tuliptree, iris, and bouncing-bet, which are exceptions noted in the work under discussion.

Because of the termination of this line of work at the Japanese beetle laboratory, no conclusions were reached as to the nature of the material or materials which caused the fruity odor of the clarified extracts.

SUMMARY

Material from 97 species and varieties of plants was analyzed for reducing-sugar content in order to determine the relationship between the sugar content of the plants and their susceptibility to attack by the Japanese beetle.

The odor imparted by the plant material to the clarified extracts, as well as the sugar content, appears to be a significant factor in immunity or susceptibility to beetle attack.

Of the plants having the clarified extracts characterized by a fruity odor, 70.9 percent of those with a reducing-sugar content of 15 to 30 mg per gram of plant material, and 46.7 percent of those with a sugar content under 15 mg were seriously infested. Of those without the fruity odor the corresponding percentages were 21.5 and 18.2.

The sucrose content could not be determined because of the coloration of the various extracts.

The nature of the substances causing the fruity odor of the clarified extracts was not determined.

HISTOLOGICAL STUDIES OF A SEEDLING DISEASE OF CORN CAUSED BY GIBBERELLA MONILIFORMIS[1]

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INTRODUCTION

Fusarium moniliforme Sheld., the imperfect stage of *Gibberella moniliformis* (Sheld.) Wineland, is found on corn (*Zea mays* L.) throughout the world (*1, 5, 10, 15*).[3] The root, stalk, and ear rots of corn with which this fungus is associated have been studied extensively in the United States and, according to the literature, they vary in economic importance in different sections of the country. The role of the fungus in the production of ear rots is generally considered more important than that in the production of seedling diseases.

Some workers, including Valleau (*18*), Holbert et al. (*8*), and Henry (*7*), considered *Fusarium moniliforme* as an active parasite in their earlier investigations. In later works, however, Koehler and Holbert (*10*) and Valleau (*19*) found it only a weak parasite. Melchers, as reported by Farrell (*6*), Melchers, and Johnston (*13, 14*), Branstetter (*2*), Johann, Holbert, and Dickson (*9*), and Leonian (*11*) also considered it a weak parasite. Edgerton and Kidder (*4*) and Sherbakoff (*17*) considered it nonparasitic. It may be concluded from the literature, therefore, that there are variations in the pathogenicity of *F. moniliforme* in different sections of the United States and even in the same locality.

Investigations in Florida (*20*) have shown that seed corn infected with this fungus produces a lower yield than noninfected seed in most cases, and that the disease causes an estimated annual loss of 5 percent of the crop. In this State, the fungus is most important as an ear-rotting organism, since it is present on a majority of the ears harvested and weakens or kills about 50 percent of the kernels on the more severely infected ears. The fungus also inhabits the soil and in some years has been observed to cause a seedling blight in the field in the course of experiments with corn diseases. Of the organisms developing on kernels while they are on the germinator, *Fusarium moniliforme* is the most prevalent. However, inoculation tests in the laboratory, greenhouse, and field with single microconidial cultures of the organism isolated from corn kernels, seedlings, stalks, and field soil showed differences in the degree of pathogenicity to seedlings and ears. Only slight infection was obtained on seedlings and ears in the field with any of the cultures, but at least 50 percent of the cultures proved to be pathogenic in the greenhouse. The others were either weakly pathogenic or nonpathogenic. Furthermore, during the course of these inoculations, some of the pathogenic cultures varied in the degree of pathogenicity, especially after remaining in culture for some time. Some of the single ascospore cultures were likewise quite vari-

[1] Received for publication June 18, 1934; issued February 1935.
[2] Grateful acknowledgment is made to Dr. W. B. Tisdale for his criticisms and suggestions.
[3] Reference is made by number (italic) to Literature Cited, p. 1015.

able in pathogenicity, as well as in growth characters. This kind of behavior has been reported by Leonian (*11*) for conidial cultures of this fungus.

Although considerable attention has been given to this disease of corn in recent years, little study appears to have been made of the pathological effects of the fungus on corn seedlings. This is probably due to the fact that the fungus is considered of little or no importance in most sections of the United States. However, since it is of importance in Florida, it seemed advisable to undertake a study of the nature of its parasitism of corn seedlings, and to supplement this with macroscopic observations bearing directly on the host-fungus relationship.

GENERAL SYMPTOMS OF GIBBERELLA INJURY TO SEEDLINGS

Although symptoms of seedlings attacked by *Fusarium moniliforme* on the germinator and in the greenhouse have been described elsewhere (*10*, *20*), the conspicuous characteristics of the diseased seedlings used in these studies are given here. Symptoms of seedling injury developing in artificially infested soil in the greenhouse are recognized by a yellowing of the leaves, stunting of the plant, and, in some cases, complete death of the leaves. Dark-brown, sunken lesions of variable size often develop on the roots and mesocotyls, and in severe cases all of these parts become brown and shrunken (pl. 1, *B*). On the rag-doll and limestone-sawdust germinators, the fungus grows rapidly, developing as a whitish to pinkish growth on the kernel, usually beginning at the tip and spreading over the seed coat, and it may extend some distance down the radicle or up the mesocotyl. On the germinator, the symptoms vary from small lesions on the radicle and mesocotyl to a complete rotting of the seedling (pl. 1, *C*, *a* to *e*); these parts do not usually become shrunken by the time the test is read, probably because of the excessive moisture present.

Except for the few cases of severe seedling blight occurring in the field, symptoms associated with young plants grown from infected seed or with seedlings attacked by the fungus present in the soil are usually not very conspicuous and cannot be distinguished easily from those resulting from other causes. However, as the season advances, the effect of seedling infection may be shown in reduced stand, vigor, and yield.

MATERIALS AND METHODS

The artificially infected corn seedlings reported upon in this paper were grown in sterilized soil in the greenhouse and in sterilized test tubes in the laboratory. They were inoculated with a single ascospore culture of the fungus which was pathogenic in previous tests. The naturally infected seedlings were obtained from the germinator or soil flats in the greenhouse. Portions of infected parts of both artificially and naturally infected seedlings were fixed at intervals and adjacent sections of each plant were plated on potato-dextrose agar. If these sections showed contamination after a period of 5 or 6 days, the corresponding fixed material was discarded. Similar portions of healthy seedlings were also fixed and stained.

The diseased material was fixed in Juel's fixative, embedded in paraffin, and sectioned 12μ to 14μ in thickness. The sections were

stained with safranine, Delafield's haematoxylin, and orange G. This combination gave a fairly good contrast between host and fungus. Healthy material was fixed in a medium chromoacetic fixative and stained with Flemming's triple stain. This combination proved to be satisfactory for the host tissue.

OBSERVATIONS AND DISCUSSION

INFECTION OF THE PLUMULE AND MESOCOTYL

The coleoptile, supported on either side by a vascular bundle (pl. 2, *A*, *b*), protects the young plumule from mechanical injury and to some extent from fungous invasion until the young shoot emerges from the soil. However, when the plumule breaks through the coleoptile, the whole shoot is readily attacked by *Gibberella moniliformis*. On the germinator a large percentage of the shoots are completely rotted soon after they break through the pericarp of the kernel or before they break through the coleoptile (pl. 1, *C*, *b*, and *c*).

Material fixed and sectioned a few days after inoculation and after natural infection appeared showed that after the fungus has penetrated the coleoptile it soon fills the spaces between the leaves (pl. 3, *B*) and from these spaces it invades the leaves, destroying the cells as it advances (pl. 2, *B*, *c*). When plumules of seedlings are attacked while on the germinator, infection usually starts in the vicinity of the first node, advances up the mesocotyl, and eventually enters the plumule. When the fungus is already present in the dry kernel, it may attack and kill the young shoot before the kernel germinates. However, where the fungus occurs on the surface of the kernel, the young plumule is not attacked until after germination. Plumules of healthy seedlings emerging from inoculated soil in the greenhouse and from inoculated tubes in the laboratory may be readily attacked by the fungus. When plumules were inoculated at the point of emergence from the coleoptile, the leaves were readily invaded by the fungus (pl. 3, *A*). In cases where the mesocotyl is severely infected, the main source of food supply coming from the primary and secondary radicles is cut off and, naturally, the plumule dies without having been invaded (pl. 1, *B*, *a*), even though it may remain alive for some time by the aid of the adventitious roots at the second node.

Since Pearson (*16*), in her studies of the parasitism of *Gibberella saubinetii* on corn seedlings, summarized much of the literature on the methods of fungous penetration in several hosts, it is not necessary to include the literature on this subject in these present studies. She reported that some workers have concluded that fungi secrete an enzyme which softens or dissolves the epidermal cell walls; others believe that fungous penetration is entirely a mechanical process, while still others consider it is a combination of the two. It is probable that both methods are concerned in bringing about the passage of *G. moniliformis* through the cell walls of the host, as cross sections of the infected mesocotyls showed that the hyphae penetrated the epidermal wall without becoming flattened and without causing any indentation of the wall. After the hyphae have penetrated the epidermal wall, they invade the cortical cells, both intercellularly and intracellularly (pl. 4, *A*.) In advanced cases the invaded cells become plasmolized (pl. 4, *B*), resulting in dark-brown sunken lesions on the mesocotyl. The fungus is capable of entering the stele of the meso-

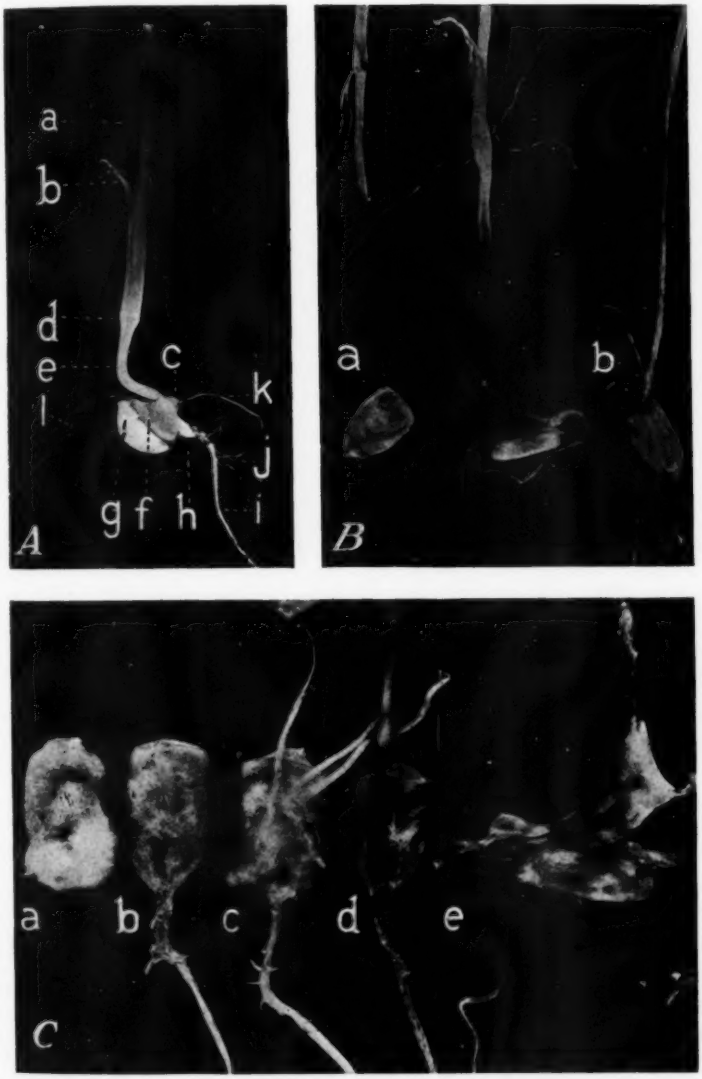
cotyl, but is usually checked, at least temporarily, by the endodermis (pl. 4, *A*, *a*). Dickson and Holbert (*3*) have shown that the endodermis and 1 or 2 layers of the pericycle in seedlings of resistant lines of corn are well suberized, even at fairly low temperatures and may thus act as a barrier against fungous invasion from the cortex into the stele. The fact that the endodermis in the mesocotyl of a corn seedling is more or less suberized probably explains why the hyphae of *G. moniliformis* was usually checked at this point.

In addition to the hyphae entering the cortex of the mesocotyl through the epidermis, it easily enters wounds produced by root emergence (pl. 5, *A* and *B*). The adventitious roots of the corn mesocotyl originate in the pericycle of the stele (pl. 5, *A*, *d*); thus, the young root growing out through the cortex ruptures the cells in its path, forming an excellent channel for invasion. However, the walls of the cells lining this rupture appear thicker and apparently become more or less suberized, thus sometimes forming a barrier against penetration. As mentioned above, invasion of the stele of the mesocotyl is often checked by the endodermis, but when the endodermal cells are disorganized by root emergence, the hyphae easily enter the stele through the young root (pl. 5, *A*, *d*). In severe cases of infection where the hyphae have entered the cortex of the mesocotyl through the epidermis and through ruptures produced by root emergence, the cells become plasmolyzed (pl. 5, *B*).

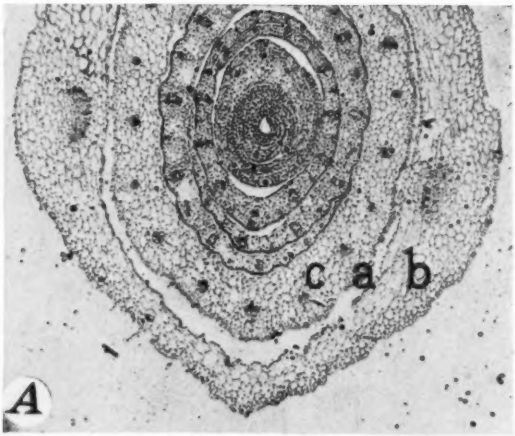
INFECTION OF THE COTYLEDONARY PLATE REGION

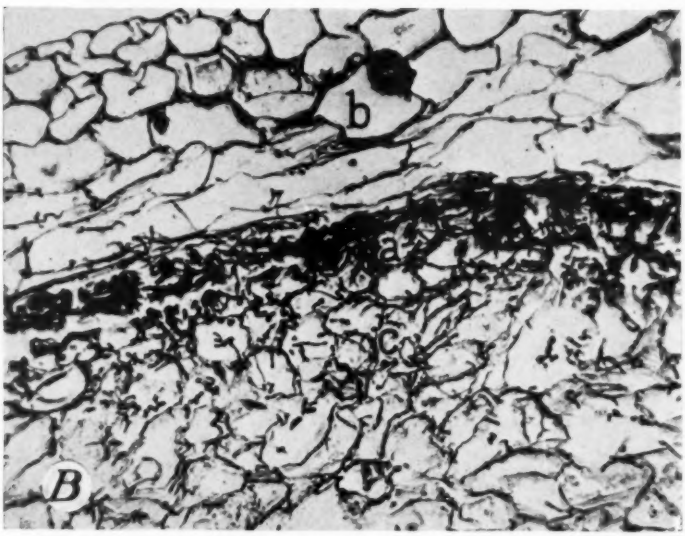
The wedging roots (pl. 6, *A*, *b*) are already present in a dormant corn kernel, and when placed under conditions for germination, these roots begin to elongate, press against the inner surface of the scutellum (pl. 6, *A*, *c*), and appear over the top of it a few days after germination (pl. 1, *A*, *l*). In pressing against the inner surface of the scutellum, they grow and widen the space betwen the scutellum and the embryonic axis by pushing the stem bud outwards, which no doubt aids the ascending axis to get clear of the seed. A little later other secondary roots (pl. 7, *A*, *d*, and *e*) push through the cortical cells at the first node opposite the point of attachment of the scutellum (pl. 1, *A*, *c*). After the stem bud (coleoptile) breaks through the pericarp, the face of the embryo becomes exposed, forming an excellent channel for invasion by the fungus.

On the germinator the presence of an active growth of the fungus on the seedling immediately after germination usually resulted in severe injury. When a seedling was inoculated in this stage, a more severe lesion resulted than when older seedlings were similarly inoculated. Material fixed at various periods after inoculation and sectioned at the cotyledonary plate region shows that the fungus readily attacks the stem in this region. The first cells invaded appear to be those located on the surface of the mesocotyl in this region. This is followed quickly by invasion of the cortical cells (pl. 6, *B*). Since a distinct endodermis is absent or has not become differentiated in the cotyledonary plate region, the fungus can easily penetrate the stele in most cases. However, in seedlings that appear to be resistant, the cells that offer resistance in the endodermal region of the cotyledonary plate are probably those of the pericycle. Seedlings similarly inoculated but sectioned when older show more severe injury. In some cases a mat of the fungus occupies the space between the inner

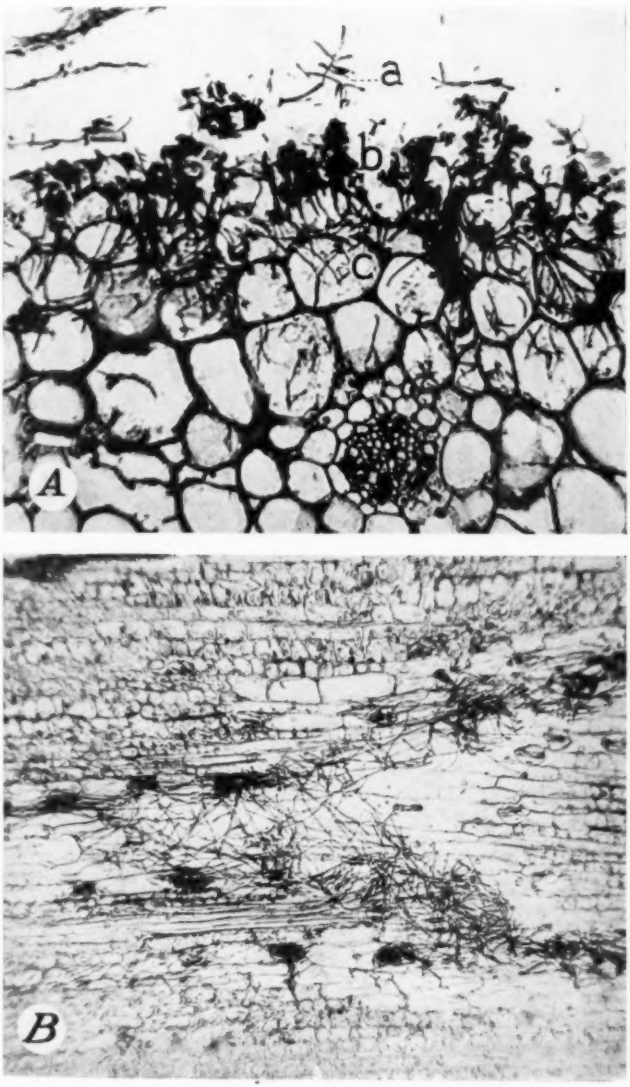


A, Normal corn seedling 8 days old: *a*, Plumule; *b*, coleoptile; *c*, first node; *d*, second node; *e*, mesocotyl; *f*, scutellum; *g*, endosperm; *h*, coleorhiza; *i*, primary radicle; *j*, lateral roots; *k*, secondary radicle; *l*, secondary radicles (wedging roots). *B*, Seedlings grown in sterilized soil inoculated with *Gibberella moniliformis* (*a*) and a seedling grown in sterilized noninoculated soil (*b*). *C*, Corn kernels after 7 days on the germinator, showing the effect of different degrees of infection with *G. moniliformis*: *a*, Severely infected and killed before germination; *b*, killed when the radicle was about 2 inches long and the plumule had just emerged; *c*, killed when the plumule was about 2 inches long; *d*, killed just before the plumule emerged from the coleoptile; *e*, killed after the plumule emerged from the coleoptile.

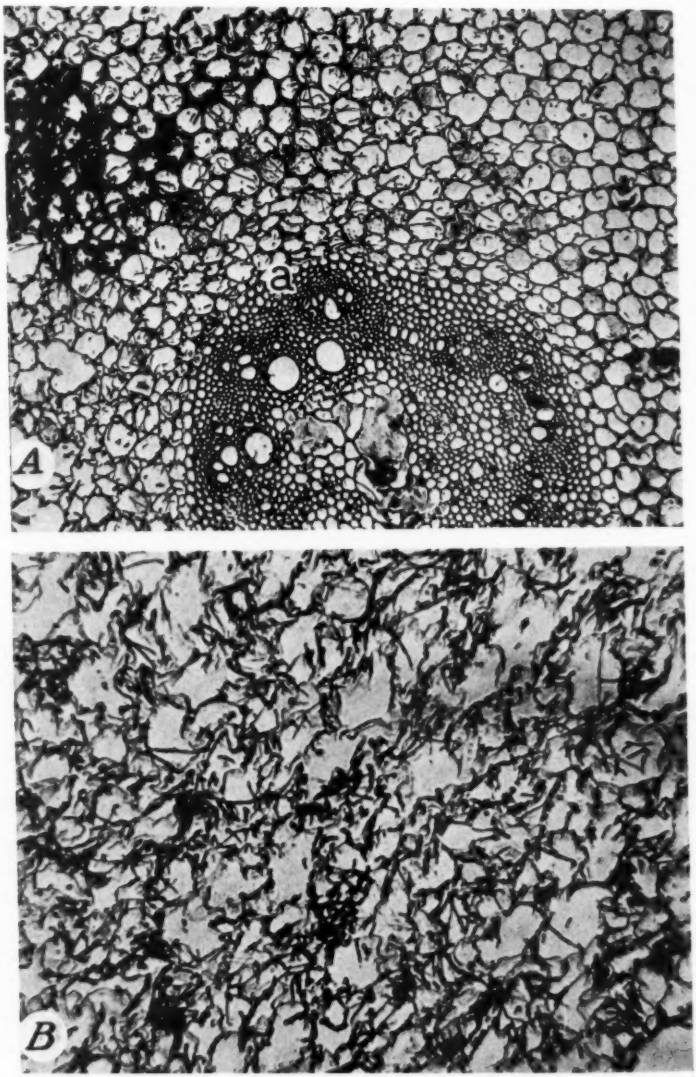




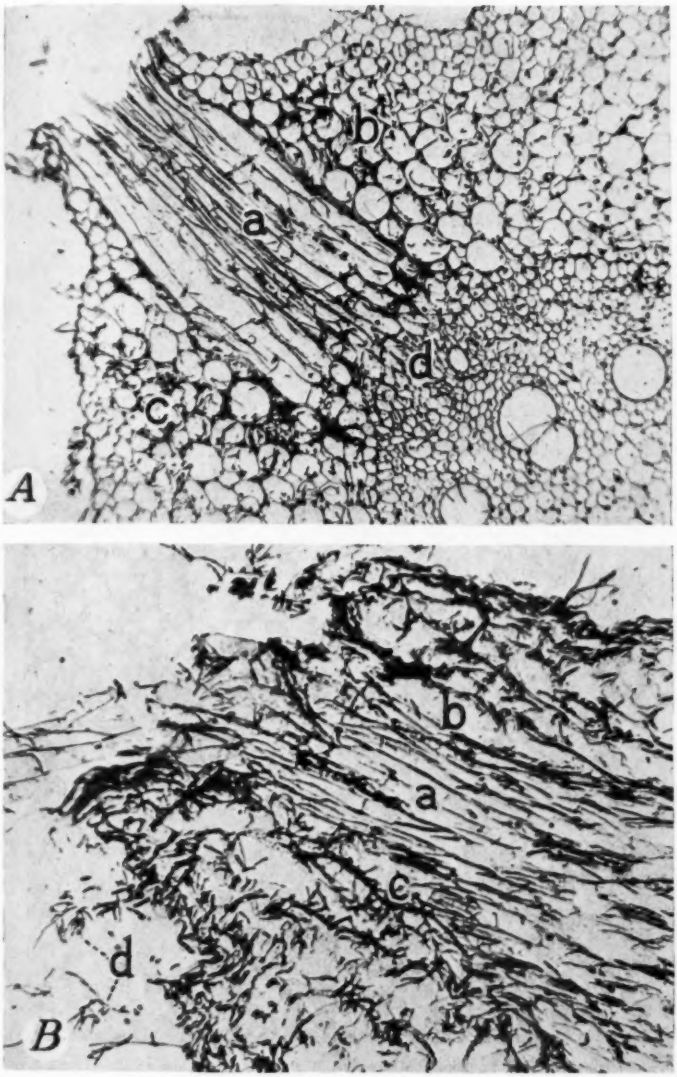
A, Cross section of a noninfected plumule just before emergence from the coleoptile: *a*, Space between coleoptile (*b*) and first leaf of plumule (*c*). ×25. *B*, Cross section of a plumule 7 days after inoculation with *Gibberella moniliformis* and just before it emerged from the coleoptile: *a*, Hyphae occupying the space between the coleoptile (*b*) and the first leaf of the plumule (*c*). Hyphae from this mat have penetrated both the coleoptile and plumule and have plasmolyzed the cells in the latter. ×225.



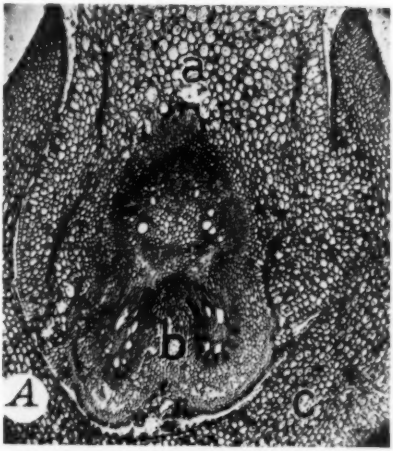
A, Portion of cross section of the first leaf of a plumule (pl. 2, *A*, *b*) inoculated at time of emergence from coleoptile with *Gibberella moniliformis: a*, Conidiophore of the fungus on the surface; *b*, hyphae entering and destroying epidermal cells; *c*, hyphae advancing into the parenchyma. ×250. *B*, Longitudinal section of a plumule naturally infected with *G. moniliformis*, showing hyphae occupying the space between the leaves (pl. 1, *A*, *a*). ×200

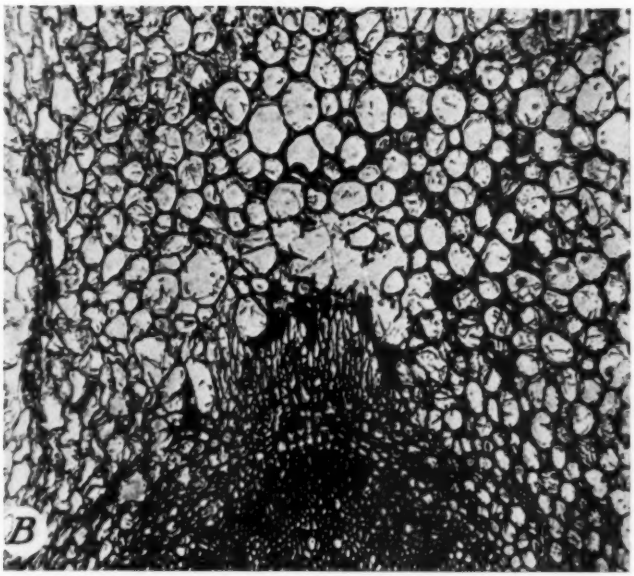


Portions of cross sections of mesocotyls naturally infected by *Gibberella moniliformis*. *A*, Hyphae are numerous in the cortex, but none were observed to pass through the endodermis (*a*). ×100. *B*, Section of a mesocotyl near the first node (pl. 1, *A*, *e*), with the cortical cells plasmolyzed by the hyphae of *G. moniliformis*. Mesocotyls in this area are especially susceptible to invasion. ×225.

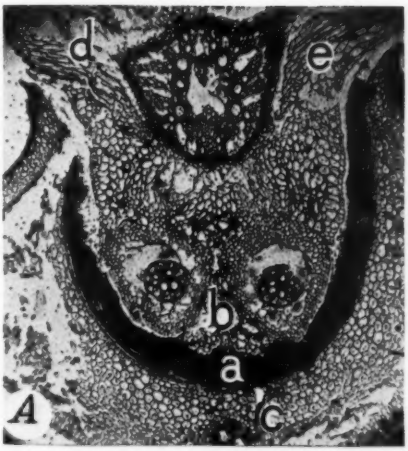


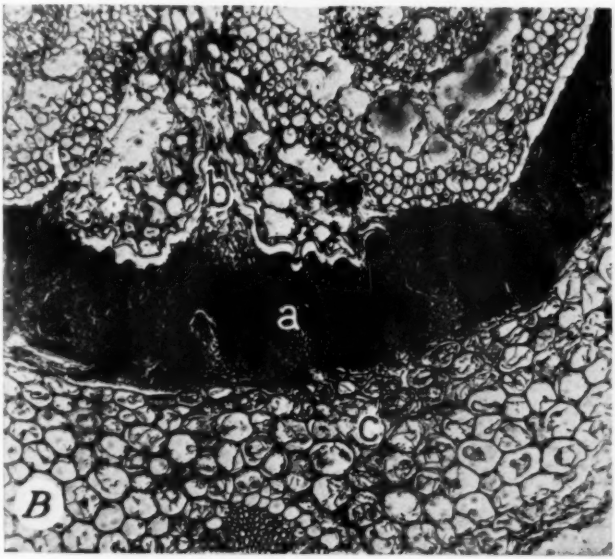
Cross sections of mesocotyls naturally infected with *Gibberella moniliformis*, showing the rupture produced in the cortex by the emergence of adventitious roots. *A*, Hyphae of *G. moniliformis* have entered the cortex (*b* and *c*) through the epidermis and through ruptures produced by root (*a*), and will enter the stele at *d*. ×150. *B*, Hyphae have entered the cortex through the epidermis and through ruptures (*b* and *c*) produced by root (*a*); conidiophores on the surface of the mesocotyl (*d*). ×200.



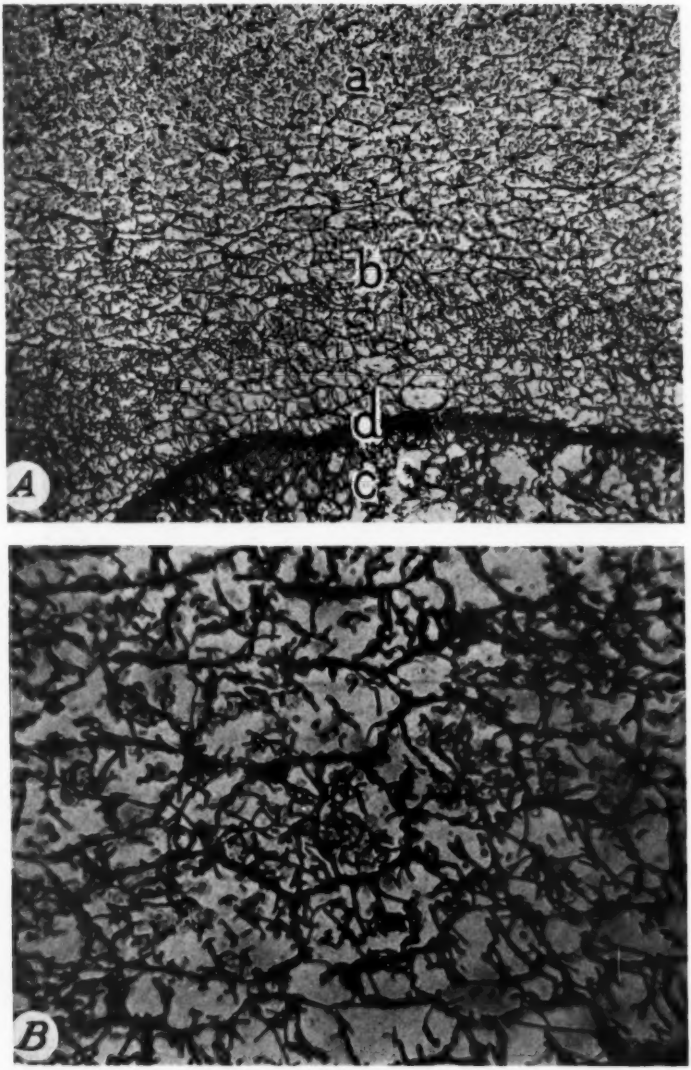


Portions of a cross section of a mesocotyl of a corn seedling in the cotyledonary plate region 6 days after inoculation with *Gibberella moniliformis* at time of germination. *A*, In this stage the hyphae are limited to the cortex (*a*) but will advance into other parts as the seedling grows; *b*, wedging roots (pl. 1, *A*, *l*) beginning to elongate and press against the inner surface of the scutellum (*c*). ×25. *B*, Enlarged portion of section *A* and (*a*) showing the hyphae in the cortical cells. ×225.

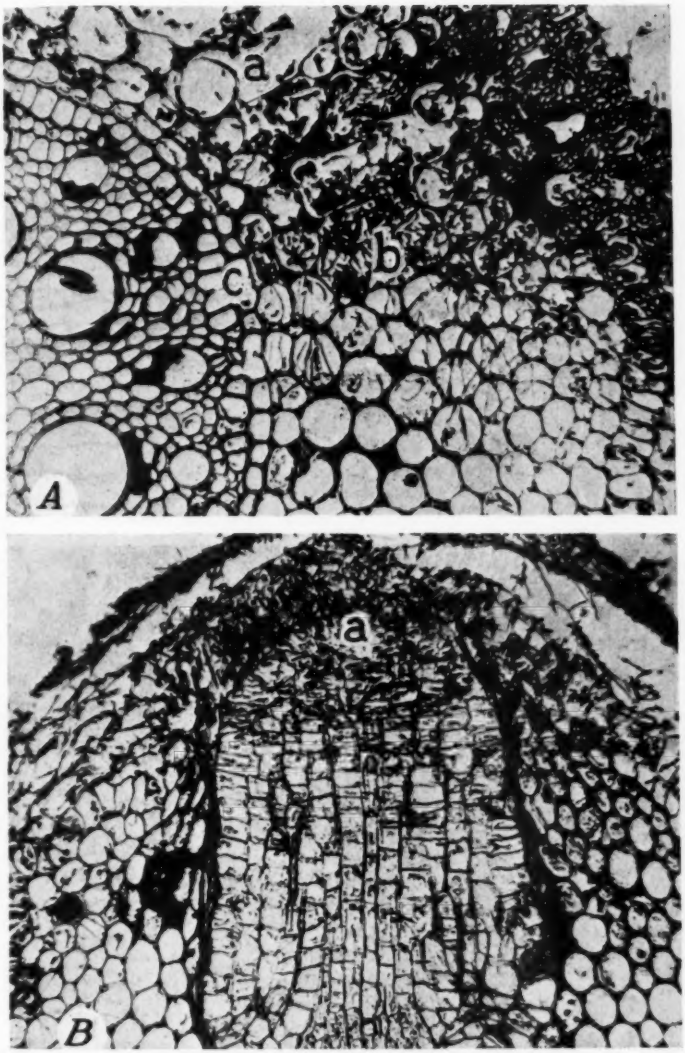




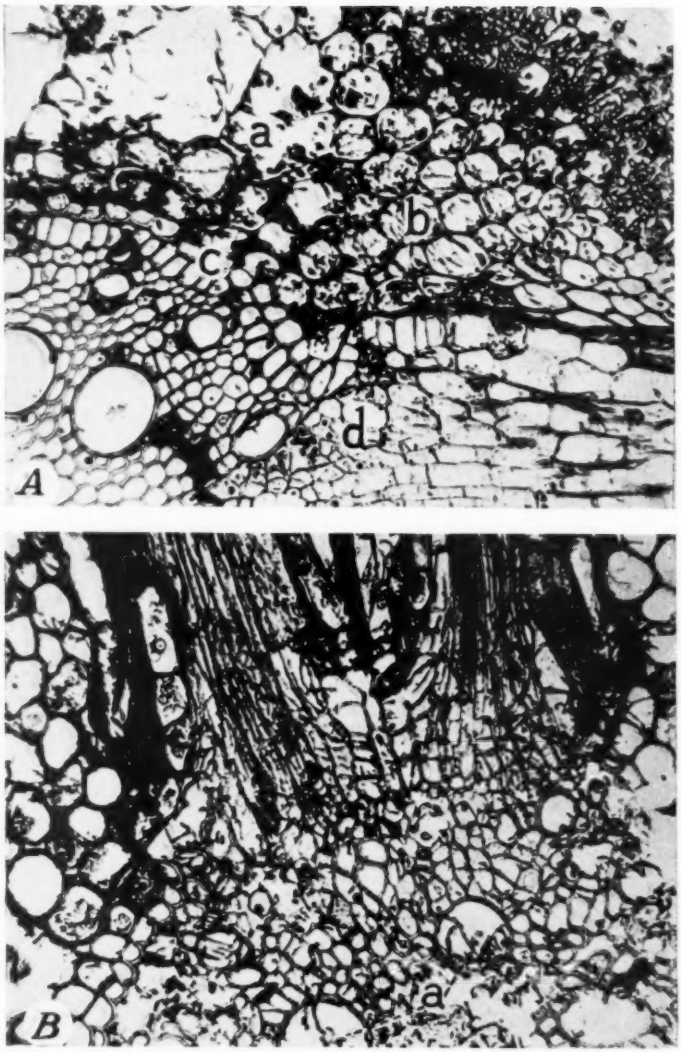
Portions of a cross section of a mesocotyl of a corn seedling in the cotyledonary plate region 12 days after inoculation with *Gibberella moniliformis* at time of germination. *A*, At this stage the hyphae have penetrated practically all parts of the seedling. *a*, *G. moniliformis* occupying the space between the secondary radicles (wedging roots) of the embryonic axis (*b*) and the scutellum (*c*). Hyphae from this mat have penetrated both the mesocotyl and the scutellum. Other secondary roots (*d* and *e*) push through the cortex at the first node (pl. 1, *A*, *b*). ×30. *B*, Enlarged portion of section *A*. ×100.



Portion of a cross section of an endosperm of a corn seedling 8 days old naturally infected with *Gibberella moniliformis*. *A*, The hyphae have destroyed most of the cells in the scutellum (*c*), formed a mat along the epithelium (*d*), penetrated the endosperm at *b*, and are advancing into the area *a*. ×100. *B*, Enlarged portion of the foregoing section at *b*, showing the hyphae in detail. ×260.



Root penetration by *Gibberella moniliformis*. *A*, Hyphae have entered the primary radicle through the epidermis and have invaded the cortex (*a* and *b*) but have been checked by the endodermis (*c*) from entering the stele. ×225. *B*, Hyphae have entered the primary radicle through lateral root tip (*a*), and through rupture produced by this root. ×225.



Root penetration by *Gibberella moniliformis*. *A*, Hyphae have entered the primary radicle through the epidermis, invaded the cortex (*a* and *b*), and been prevented by the endodermis (*c*) from entering the stele, but they will eventually enter it through the lateral root (*d*). ×225. *B*, Hyphae have entered the radicle through a lateral root, penetrated the stele, and are now within the vessels (*a*). ×225.

surface of the scutellum and the embryonic axis (pl. 7, *B*, *a*). Hyphae from this mat have penetrated the cortex of the axis and the scutellum and, from the scutellum, will advance into the endosperm.

The fungus may be dormant in dried, naturally infected kernels, but when the kernels are placed under conditions suitable for germination, the fungus starts developing immediately and in some cases will invade all parts in 6 days. This coincides with the results of Manns and Adams (*12*). The storage tissues of the scutellum and endosperm are particularly susceptible to invasion. Naturally infected kernels fixed and sectioned through the cotyledonary plate region shortly after germination showed that the fungus was well established in these tissues (pl. 8, *A*, *b* and *c*). In this particular seedling, the fungus apparently entered the endosperm from the scutellum, forming a mat of hyphae along the epithelium (pl. 8, *A*, *d*). It appears that the fungus has destroyed the starch grains in some of the cells of the endosperm next to the scutellum (pl. 8, *A* and *B*) and is advancing farther into the endosperm, destroying or absorbing the cell contents as it advances (pl. 8, *A*, *a*). However, this may not be due to the fungus, since the contents of the endosperm are naturally dissolved or absorbed by the scutellum, which transmits them in solution to the growing parts of the embryo.

INFECTION OF THE ROOTS

The first evidence of germination of a corn kernel is the coleorhiza breaking through the pericarp at the distal end of the kernel. The coleorhiza extends a short distance and does not develop further (pl. 1, *A*, *h*). The primary radicle fills this extending sheath, finally breaks through it and continues to elongate (pl. 1, *A*, *i*). Although the fungus is capable of penetrating the epidermal wall of the unruptured coleorhiza, the primary radicle soon ruptures the coleorhiza, forming a channel for invasion. Pearson (*16*) has shown that *Gibberella saubinetii* penetrates the unruptured coleorhiza of a corn seedling through pluglike structures between the epidermal cells, the fungus gaining entrance into the host by dissolving the pluglike structures.

In naturally infected kernels *Gibberella moniliformis* first appeared at the distal end of the kernel, then spread over the seed coat (pl. 1, *C*, *a* and *b*). Seedlings sectioned at this point showed that the fungus did not usually enter the plant until after the primary radicle ruptured the coleorhiza. Penetration of the tip of the primary radicle was also evident at this time. As mentioned before, the rupture produced by the young stem bud breaking through the pericarp also affords an excellent channel for invasion in the germinating kernel.

Seedlings inoculated with *Gibberella moniliformis* at the point of emergence of the primary radicle and sectioned 6 days later showed that the fungus had grown some distance down the radicle, penetrating it through the epidermis and invading the cortical cells (pl. 9, *A*, *a* and *b*) and was inhibited by the endodermis from entering the stele (pl. 9, *A*, *c*). The endodermis covering the stele in the radicle is apparently more suberized than that in the mesocotyl, since no hyphal penetration of the endodermis in the radicle was observed.

Similar to the adventitious roots of the mesocotyl, the lateral roots of the primary radicle grow out through the cortex, rupturing the

cortical cells in its path and opening a channel for invasion. The young root tips of these lateral roots are very susceptible to invasion (pl. 9, *B*, *a*). The fungus invades these roots (pl. 10, *A*, *d*) and finally enters the stele and the vessels (pl. 10, *B*, *a*). Although the fungus is capable of entering the rupture produced by the emergence of the lateral roots and invading the cortex of the radicle, the cell walls lining this rupture are apparently more suberized than those under similar conditions in the mesocotyl; thus they form a more effective barrier against penetration.

SUMMARY

Gibberella moniliformis is a prevalent corn seed parasite in Florida, as determined by germination tests and by reduced yields in the field. Inoculation experiments in the laboratory, greenhouse, and field have shown that single-spore cultures varied in degree of pathogenicity.

Pathogenic strains of the fungus readily attack corn seedlings in the greenhouse, either when they are naturally or artificially inoculated.

Symptoms following inoculations in the greenhouse are a yellowing of the leaves, retardation in growth, dark-brown lesions on the mesocotyls and roots and, in cases of severe infection, more or less complete drying of the leaves, followed by the death of the seedling. On the germinators, the fungus growth on the kernels and seedlings is quite visible, and injury to the seedlings varies from small lesions on the radicle and mesocotyl to a complete rotting of the seedling. Injury in the field is not easily differentiated in the seedling stage, but the effect of such injury is evident later in the season by reduced stand, vigor, and yield.

The fungus enters the plumule where it breaks through the apex of the coleoptile, through the mesocotyl, and directly through the coleoptile. It grows rapidly in the spaces between the leaves, finally penetrating the leaves and destroying the cells.

Invasions of the mesocotyl result from direct penetration of the epidermis, and through ruptures in the cortex produced by the emergence of adventitious roots. Dark sunken lesions develop on the mesocotyl as a result of the cortical cells becoming plasmolyzed.

The fungus may enter the cotyledonary plate region through the natural opening produced by the stem bud breaking through the pericarp and advance into the storage tissues (scutellum and endosperm). However, it may invade these tissues first by entering through the rupture produced by emergence of the coleorhiza.

The first evidence of infection in the germinating kernel usually shows up at the distal end on the coleorhiza and primary radicle. The fungus may enter the primary radicle directly through the epidermis, but it usually enters through ruptures produced in the cortex by the emergence of lateral roots.

The endodermis surrounding the stele in the mesocotyl and primary radicle acts as a barrier against penetration, its effectiveness depending upon the degree of suberization. However, the fungus easily enters the stele of the mesocotyl and primary radicle through the adventitious and lateral roots, respectively.

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THE UTILIZATION OF AGRICULTURAL BYPRODUCTS IN THE PRODUCTION OF PROPIONIC ACID BY FERMENTATION[1]

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INTRODUCTION

The production of propionic acid from agricultural byproducts by the propionic acid bacteria offers commercial possibilities which have not been adequately investigated.

Sherman and his coworkers (*3, 5, 6*)[2] have considered the commercial merits of the propionic fermentation, especially with reference to the utilization of the lactose of whey and milk. They obtained the most satisfactory results when the propionic acid bacteria were grown in association with other organisms, principally members of the lactic acid and proteus groups. However, under their most satisfactory conditions the fermentation was rather slow. They indicated that industrial application was doubtful.

Van Niel (*1*) has shown that the propionic acid bacteria are limited in their utilization of nitrogen from various sources. Vigorous fermentations were obtained only when relatively high concentrations of yeast were used. The ratio of propionic acid to acetic acid as well as the yield of volatile acids was found to vary with the concentration of yeast extract.

Of interest from an industrial standpoint is van Niel's isolation of a starch-fermenting species, *Propionibacterium technicum*. He experimented briefly with this strain, but the fermentation obtained was slow. Van Niel suggested a method of speeding up the propionic acid fermentation. He observed that with many strains the propionic acid bacteria settle to the bottom of the flask after fermentation is complete, leaving a relatively clear supernatant liquid. By removing the liquid above the bacteria and adding sterile medium a 2-percent sugar solution was completely fermented within 48 hours.

Wilson, Fred, and Peterson (*7*) worked on the fermentation of malted corn, blackstrap molasses, and hydrolyzed starch by several strains of propionic acid bacteria grown in association with *Lactobacillus casei*. The fermentation of molasses and hydrolyzed starch was practically complete in 8 to 10 days. The yield of volatile acids was low, however, ranging from 45 to 59 percent of the fermented sugar. Of some industrial significance is the fact that residues of the acetone-butyl alcohol fermentation were found to serve as a source of nitrogen in these fermentations.

Recently a number of patents have been granted in the United States pertaining to the production of propionic acid by fermentation, indicating that commercial production is under investigation.

[1] Received for publication May 28, 1934; issued February 1935. Journal Paper No. J. 172 of the Iowa Agricultural Experiment Station. Project no. 65.

[2] Reference is made by number (italic) to Literature Cited, p. 1024.

This brief survey of the literature indicates that there are three problems that require solution before extensive industrial production will be feasible.

(1) *The nitrogen requirements.*—Yeast extract is the only satisfactory source of nitrogen which has been reported for this group. Utilization of residues from the butyl-acetone fermentation in which pure cultures were used has not been reported. A cheap and satisfactory source of nitrogen is essential for commercial production.

(2) *The carbohydrate.*—The most satisfactory source of carbohydrates for this fermentation has not been definitely established. A knowledge of the number of materials which can be used satisfactorily and the merits of each is essential.

(3) *The period of fermentation.*—The prolonged period of fermentation is one of the present difficulties in the way of commercial utilization of the propionic acid bacteria.

The object of this investigation was: (1) To determine the utilization made of natural carbohydrate materials as well as carbohydrate by-products of industrial processes by *Propionibacterium arabinosum;* (2) to determine the rate of fermentation of these materials; and (3) to determine the effect of different sources of nitrogen.

PRELIMINARY EXPERIMENTS

Preliminary investigations indicated that *Propionibacterium arabinosum* possesses strong fermentative powers. Table 1 shows typical results. Fermentation of glucose was vigorous; a 3-percent solution was utilized in 9 days under favorable conditions. Of special note is the high yield of propionic acid. The fermentation of starch, although active, was much slower than that of glucose.

TABLE 1.—*Production of volatile acids (percentage by weight) by fermentation of glucose or starch*

[Medium: Yeast extract, carbohydrate (3 percent) and $CaCO_3$ (3 percent); glucose, 9-day fermentation; starch, 14-day fermentation at 30° C.]

Carbohydrate	Yeast extract [1]	Carbohydrate fermented	Volatile acid per liter of medium	Fermented sugar as volatile acids	Volatile acid as—		
					Propionic	Acetic	Formic
	Percent	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*
Glucose	1	53.0	8.7	54.6	84.7	12.8	2.5
	5	98.9	16.9	56.9	82.7	17.3	0
	10	97.4	19.5	66.7	80.0	20.0	0
Starch	10	57.6	11.1	64.6	73.4	23.0	3.6
	10	50.3	9.9	65.6	73.0	26.0	1.0

[1] Water extract of a quantity of dried yeast equivalent to the indicated percentage by weight of the fermentation medium.

Further study indicated that corn gluten serves as a source of nitrogen for the propionic acid bacteria. In preliminary fermentations of glucose in which gluten was used as a source of nitrogen, yields were comparable to those obtained with yeast.

These preliminary experiments indicated that a nitrogen source had been found suitable for the growth of the propionic acid bacteria. Further investigation concerning the industrial application of this organism seemed of value.

PROCEDURE AND METHODS

Approximately, 1,600 cc of medium was used in each fermentation. Three fermentations of each carbohydrate were conducted. The first contained no source of nitrogen other than that present in the raw material; to the second 100 g of dried yeast was added, and to the third 100 g of corn gluten. Fifty grams of $CaCO_3$ was used as buffer in each flask. In those fermentations in which hydrol, whey, milk, or blackstrap molasses served as the raw material, a sufficient quantity was added to supply 80 g (5 percent) of carbohydrate. The carbohydrate content was 48 g (3 percent) in the corn-meal, potato, and artichoke media. Fermentation of yeast (100 g) and of corn meal (100 g) with no addition of carbohydrate material was also run.

The media were autoclaved for 1 hour at 20 pounds pressure and were still sterile after 3 days' trial incubation.

Inoculations were made by using 60 cc of 7-day cultures of *Propionibacterium arabinosum* (strain described by Werkman and Brown (*4*)) grown in a medium consisting of 0.5 percent of Bacto yeast extract, 2 percent of glucose, and 1 percent of $CaCO_3$. The fermentations were incubated at 30° C. No attempt was made to control the degree of anaerobiosis. Cotton plugs were used as stoppers. The media were thoroughly shaken twice daily to aid the buffer action of the $CaCO_3$.

Eight hundred cubic centimeters of the fermented medium was removed from each flask after 7 days' incubation. The remaining 800 cc was fermented during an additional 7-day period. The cultures were checked microscopically for purity by the Gram stain. The volatile acids were determined by the partition method (*2*) as described by Osburn, Wood, and Werkman.

EXPERIMENTAL RESULTS

The yields of volatile acids obtained from the raw materials are summarized in table 2. The results of the fermentation of yeast and of gluten meal are presented in table 3.

The yield is calculated on the basis of 1 l of medium. The volatile acids in grams per 100 g of carbohydrate were determined on the basis of carbohydrates present in the raw material. The total carbohydrate utilized was not determined. It is apparent that these calculations are only comparable for fermentations within the groups containing 3 percent (or 5 percent) of added carbohydrate. No correction has been made for carbohydrates which may have been added with the nitrogen source, since the primary object was to determine the amount of volatile acids obtainable from a given quantity of material in a definite period. Fermentation of the nitrogen source, particularly of gluten meal, indicates the presence of considerable quantities of fermentable material. In the case of corn gluten this is largely cornstarch.

Hydrol and whey were the most satisfactory of the carbohydrate materials examined. The theoretical yield of volatile acids in the propionic acid fermentation is approximately 75 g per 100 g of glucose utilized. The recovery of 69 g in the hydrol-yeast medium indicates that utilization of the 5 percent of carbohydrate was practically complete in 14 days. It is interesting to note that in fermentations

in which pure cultures of propionic acid bacteria were used there have been obtained in this laboratory substantial yields of the nonvolatile acids, succinic and lactic. It is thus possible that the sugar utilization was more complete than is indicated by the yields of volatile acids.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*

NO SOURCE OF NITROGEN—14-DAY FERMENTATION

Byproducts and the acids formed	Volatile acid produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*
Corn meal: [1]				
Propionic				
Acetic				
Formic				
Total	21.4			
Artichoke: [1]				
Propionic	85.8	6.34	75.6	21.2
Acetic	33.1	1.99	23.7	6.6
Formic	1.3	.06	.7	.2
Total	120.2	8.39		28.0
Potato: [1]				
Propionic	39.1	2.89	71.5	9.6
Acetic	13.4	.80	19.8	2.7
Formic	7.6	.36	8.7	1.2
Total	60.1	4.05		13.5
Hydrol: [2]				
Propionic	24.4	1.81	74.1	3.6
Acetic	5.0	.30	12.3	.6
Formic	7.4	.33	13.5	.7
Total	36.8	2.44		4.9
Whey: [2]				
Propionic	31.2	2.39	63.1	4.8
Acetic	18.6	1.12	29.6	2.2
Formic	6.0	.27	7.3	.5
Total	55.8	3.78		7.5
Milk: [2]				
Propionic	47.2	3.49	74.6	7.0
Acetic	19.6	1.14	24.4	2.3
Formic	1.0	.04	1.0	.1
Total	67.8	4.67		9.4
Blackstrap: [2]				
Propionic	84.0	6.22	80.3	12.5
Acetic	15.8	.94	12.2	1.9
Formic	12.6	.58	7.5	1.1
Total	112.4	7.74		15.5

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydtate.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*—Continued

GLUTEN ADDED AS SOURCE OF NITROGEN

Byproducts and the acids formed	7-day fermentation				14-day fermentation			
	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*	*Cubic centimeters*	*Grams*	*Percent*	*Grams*
Corn meal:[1]								
Propionic	95.3	7.05	70.2	23.5	154.8	11.46	70.8	38.2
Acetic	48.8	2.93	29.1	9.7	78.2	4.68	29.0	15.6
Formic	1.5	.07	.7	.2	.9	.04	.2	.1
Total	145.6	10.05		33.5	233.9	16.18		53.9
Artichoke:[1]								
Propionic	110.0	8.15	70.5	27.1	147.4	10.91	66.8	36.4
Acetic	55.5	3.33	28.9	11.1	87.1	5.21	31.9	17.4
Formic	1.7	.07	.6	.5	4.7	.22	1.3	.7
Total	167.2	11.55		38.7	239.1	16.34		54.5
Potato:[1]								
Propionic	50.2	3.71	65.3	12.4	83.0	6.14	67.6	20.5
Acetic	32.2	1.93	34.0	6.4	47.6	2.86	31.4	9.5
Formic	.9	.04	.7	.1	2.0	.09	1.0	.3
Total	83.3	5.68		18.9	132 6	9.09		30.3
Hydrol:[2]								
Propionic	121.5	8.99	70.7	18.0	222.2	16.46	81.6	32.9
Acetic	61.9	3.72	29.3	7.4	59.4	3.56	17.6	7.2
Formic	(3)				3.7	.17	.8	.3
Total	183.4	12.71		25.4	285.4	20.19		40.4
Whey:[2]								
Propionic	61.6	4.50	74.7	9 0	143.3	10.61	71.7	21 2
Acetic	24.5	1.47	24.3	3.0	66.6	4.00	27.2	8.0
Formic	1.4	.06	1.0	.1	3.2	.16	1.1	.3
Total	87.5	6.03		12.1	213.1	14.77		29.5
Milk:[2]								
Propionic	69.2	5.12	70.3	10.2	175.0	12.93	75.5	25.9
Acetic	34.9	2.09	28.8	4.2	68.5	4.12	24.0	8.2
Formic	1.5	.07	.9	.1	2.2	.10	.5	.2
Total	105.6	7.28		14.5	245.7	17.15		34.3
Blackstrap:[2]								
Propionic	209.5	15.51	85.0	31.0	311.7	23.03	82.1	46.1
Acetic	43.2	2.59	14.2	5.2	80.1	4.82	17.1	9.6
Formic	3.3	.15	.8	.3	5.1	.24	.8	.5
Total	256.0	18.25		36.5	396.7	28.10		56.2

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydrate.
[3] Trace.

TABLE 2.—*Production of volatile acids from various byproducts by Propionibacterium arabinosum (calculated on a basis of 1,000 cc of medium) without the addition of a nitrogen source, and with yeast or gluten added as nitrogen sources*—Continued

YEAST ADDED AS SOURCE OF NITROGEN

Byproducts and the acids formed	7-day fermentation				14-day fermentation			
	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate	Volatile acids produced expressed as normal acid	Portion by weight of total acid		Volatile acid produced per 100 g of carbohydrate
	Cubic centimeters	*Grams*	*Percent*	*Grams*	*Cubic centimeters*	*Grams*	*Percent*	*Grams*
Corn meal:[1]								
Propionic	42.4	3.13	71.8	10.4	73.7	5.45	73.0	18.2
Acetic	19.7	1.18	27.1	4.0	32.8	1.97	26.3	6.6
Formic	1.1	.05	1.1	.2	1.1	.05	.7	.2
Total	63.2	4.36		14.5	107.6	7.47		24.9
Artichoke:[1]								
Propionic	113.3	8.38	70.7	28.0	129.2	9.56	68.3	31.9
Acetic	55.1	3.31	28.0	11.0	70.6	4.24	30.2	14.1
Formic	3.3	.15	1.3	.5	4.5	.21	1.5	.7
Total	171.7	11.84		39.5	204.3	14.01		46.7
Potato:[1]								
Propionic	53.3	3.95	73.7	13.2	78.7	5.82	71.2	19.4
Acetic	23.4	1.40	26.3	4.7	38.9	2.33	28.5	7.8
Formic	[3]				.6	.03	.3	.1
Total	76.7	5.35		17.8	118.2	8.18		27.3
Hydrol:[2]								
Propionic	242.3	17.93	72.8	35.9	338.9	25.10	72.7	50.2
Acetic	108.0	6.48	26.2	13.0	154.0	9.24	26.8	18.5
Formic	5.0	.23	1.0	.4	4.0	.18	.5	.3
Total	355.3	24.64		49.3	496.9	34.52		69.0
Whey:[2]								
Propionic	140.1	10.37	72.9	20.7	350.9	25.95	76.9	51.9
Acetic	62.8	3.78	26.5	7.6	128.6	7.71	22.8	15.4
Formic	1.8	.08	.6	.1	1.9	.09	.3	.2
Total	204.7	14.23		28.4	481.4	33.75		67.5
Milk:[2]								
Propionic	204.2	15.11	75.1	30.2	303.5	22.47	76.4	44.9
Acetic	80.7	4.84	24.1	9.7	113.8	6.83	23.2	13.7
Formic	3.4	.16	.8	.3	2.5	.11	.4	.2
Total	288.3	20.11		40.2	419.8	29.41		58.8
Blackstrap:[2]								
Propionic	207.4	15.36	72.6	30.7	256.7	18.99	75.3	38.0
Acetic	85.6	5.13	24.3	10.2	100.2	6.01	23.8	12.0
Formic	14.4	.66	3.1	1.3	5.1	.23	.9	.5
Total	307.4	21.15		42.2	362.0	25.23		50.5

[1] Medium contained 3 percent carbohydrate.
[2] Medium contained 5 percent carbohydrate.
[3] Trace.

TABLE 3.—*Production of volatile acids (calculated on the basis of 1,000 c c of medium) by the fermentation of gluten and yeast for 7- and 14-day fermentations*

Acids	Production for 7-day gluten fermentation			Production for 14-day gluten fermentation			Production for 7-day yeast fermentation			Production for 14-day yeast fermentation		
	As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid		As normal acid	Portion by weight of total acid	
	Cubic centimeters	*Grams*	*Per cent*	*Cubic centimeters*	*Grams*	*Per cent*	*Cubic centimeters*	*Grams*	*Per cent*	*Cubic centimeters*	*Grams*	*Per cent*
Propionic	22.0	1.63	57.2	39.5	2.92	54.5	19.9	1.48	74.7	21.2	1.58	77.5
Acetic	17.6	1.05	36.9	33.4	2.01	37.4	8.0	.48	24.3	6.0	.36	17.6
Formic	3.6	.17	5.9	9.4	.43	8.1	.7	.02	1.0	2.1	.10	4.9
Total	43.2	2.85	------	82.3	5.36	------	28.6	1.98	------	29.3	2.04	------

It is evident that there was no source of nitrogen in any of the raw materials examined suitable to support a vigorous propionic fermentation. Comparison of yeast and gluten as nitrogen sources for the propionic acid fermentation is made difficult by the large quantity of acids obtained in the fermentation of the gluten meal (table 3). The large yield of acids obtained in the fermentation of blackstrap molasses indicates, however, the value of corn gluten as a source of nitrogen. Further study will be required to explain the low yields obtained from carbohydrates with gluten meal as a nitrogen source as compared with yeast. Further study will be required to explain the low yields obtained from carbohydrates with gluten meal as a nitrogen same as compared with yeast.

The percentages of propionic acid obtained in the fermentation of raw materials were somewhat lower than those found in glucose fermentations.

Results which are closely related to those described above have been obtained in this laboratory in the case of propionic acid fermentations in which steep water was utilized as a nitrogen source. Data obtained with glucose-steep water fermentations with *Propionibacterium arabinosum* are summarized in table 4. Similar data have been obtained with other strains of propionic acid bacteria. These results are in most respects similar to those obtained with glucose-yeast extract fermentations (table 1). Concentrations of 5 to 10 percent of steep water appear adequate to fulfill the nitrogen requirements of the propionic acid bacteria. Since steep water is a by-product resulting from the commercial production of corn sugar, it seems possible that it can be utilized as a nitrogen source in commercial fermentations by the propionic acid bacteria. Further studies with larger fermentations in which carbohydrate materials with commercial possibilities are used will be necessary to determine the value of steep water as a nitrogen source. The results reported indicate, however, that steep water is a potential and cheap source of nitrogen for the propionic acid fermentation.

TABLE 4.—*Production of volatile acids (percentage by weight) by 14-day fermentation at 30° C. of glucose (3 percent), $CaCO_3$ (2 percent), and various percentages by weight of steep water*

Steep water used	Sugar fermented	Volatile acid produced per 1,000 cc of medium	Fermented sugar as volatile acids	Volatile acid as—	
				Propionic	Acetic
Percent	*Percent*	*Grams*	*Percent*	*Percent*	*Percent*
0.5	58.3	7.8	44.7	84.5	15.5
1.0	66.4	12.9	64.7	84.1	15.9
5.0	89.4	18.4	68.4	80.5	19.5
10.0	96.2	21.6	74.8	79.6	20.4

SUMMARY AND CONCLUSIONS

The utilization of hydrol, whey, milk, blackstrap molasses, artichoke tubers, and potatoes in the production of propionic acid by fermentation has been determined together with an evaluation of corn gluten meal and yeast as nitrogen sources. A summary is presented of investigations on the fermentation of glucose in which steep water was used as a source of nitrogen.

The results of the experiments have led to the following conclusions:

Hydrol, whey, milk, blackstrap molasses, artichoke tubers, potatoes, and corn meal contain carbohydrates which are utilized by *Propionibacterium arabinosum*. Hydrol and whey are the most satisfactory of the materials tested.

Corn gluten meal and steep water serve as sources of nitrogen for the propionic acid bacteria.

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EFFECT OF DYES ON YEAST FERMENTATION AS INFLUENCED BY HYDROGEN-ION CONCENTRATION[1]

By ESTHER ADAMS, *instructor in biology, Trenton Junior College*, and WILLIAM J. ROBBINS, *botanist, Missouri Agricultural Experiment Station*

INTRODUCTION

The effect of hydrogen-ion concentration on the toxicity of various organic and inorganic compounds has been studied by a number of investigators. In general the results have shown that the toxicity of anions is increased by increasing the hydrogen-ion concentration and that of cations is increased by decreasing the hydrogen-ion concentration, provided the reaction does not affect the solubility of the compound studied. The earlier literature on this subject has been reviewed elsewhere (*11*).[2] The present investigation is concerned with the influence of hydrogen-ion concentration upon the effect of certain acid and basic dyes on yeast fermentation. Von Euler and Florell (*3*) in studying the effect of dyes on yeast found that in the presence of 0.05 percent of the acid dye ponceau 3R, fermentation was checked a little more at pH 3.8 than at pH 6.1, and at a 0.5 percent concentration of the dye, fermentation was checked considerably more at pH 2.2 to 2.4 than at pH 6.1 to 6.3. Hydrogen-ion concentration had little effect upon the toxicity of the basic dye, methylene blue. No other investigations on the influence of hydrogen-ion concentration on the toxicity of dyes to yeast have been noted by the authors.

INVESTIGATION

PROCEDURE

The acid dyes, eosin (Schultz no. 512) and rose bengale (Schultz no. 520); and the basic dyes, dahlia (Schultz no. 451), safranine (Schultz no. 584), and brilliant green (Schultz no. 428) were used (*12*). A suspension of yeast, *Saccharomyces ceriviseae*, was prepared by suspending a cake of Fleischmann's yeast in 50 cc of redistilled water. Twelve drops of this suspension were added to each fermentation tube (25 cc of solution). The fermentation tubes were incubated at 32° C. and the percentage of gas produced was measured at intervals by means of the Frost gasometer. Redistilled water and Pyrex glassware were used throughout.

In the first experiment performed, buffer mixtures were prepared by titrating $\frac{N}{40}$ Na_2HPO_4 with $\frac{M}{10}$ H_3PO_4 and diluting to constant volume so that the final solutions contained $\frac{N}{50}$ sodium. To these buffer mixtures glucose sufficient to make the concentration 4 percent was added. A uniform dilution of the dye studied was added to the various buffer mixtures, which were placed in fermentation tubes, inoculated with yeast, incubated at 32° C., and the gas production noted. By following this procedure it was found that initial reactions of from pH 4.3 to pH 8.0 had little effect upon the amount of gas

[1] Received for publication Aug. 15, 1934; issued February 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 1031.

produced after 8, 20, and 34 hours in the buffer mixtures containing sugar but no dye. Some retardation of fermentation was observed at pH 3.1 and 3.6 and at pH 8.8. These results agree in general with those of Von Euler and Heintze (*4, 5*), who found little difference in fermentation at reactions of from pH 4.0 to pH 7.0, and with those of Hägglund and Rosengvist (*6*), who found that fermentation with yeast juice proceeded with practically the same velocity between pH 5.5 and pH 8.0.

If rose bengale at a dilution of 1 to 100 or 1 to 1,000 is added to the buffer mixtures containing glucose the results are as shown in figure 1. The toxicity of this acid dye is markedly increased at original pH values of 6.0 and lower. However, when this method of determining the influence of hydrogen-ion concentration on the toxicity of dyes to yeast is used, the production of carbon dioxide and other fermentation products affects the reaction of the buffer mixtures. Those solutions more alkaline than about pH 5.0 become more acid; whereas those solutions more acid than pH 5.0 are unaffected or become less acid. The original pH of the buffer mixtures does not represent, therefore, the reaction to which the yeast is subjected during the entire period of incubation.

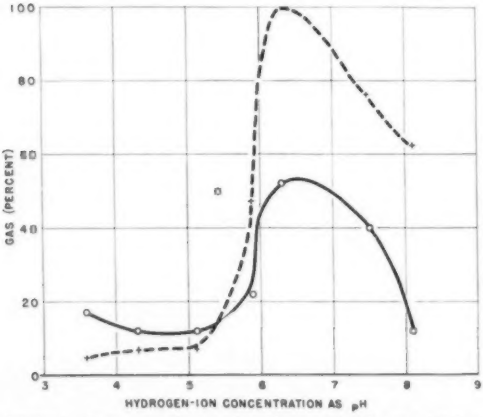

FIGURE 1.—Percentage of gas produced by yeast in 9 hours at 32° C. at various hydrogen-ion concentrations in sodium phosphate buffer mixtures containing 4 percent glucose and rose bengale, 1 to 100 (solid line) or 1 to 1,000 (dotted line). Percentage of gas plotted against original pH of solutions.

To avoid this difficulty the following procedure was used: The yeast was exposed in 150 cc Erlenmeyer flasks for 24 hours at room temperature to the dye at various hydrogen-ion concentrations in the sodium phosphate buffer mixtures which contained no sugar. At the end of 24 hours the dye and buffer mixtures were decanted and replaced by a nutrient solution composed of KH_2PO_4, 0.1 g; NH_4NO_3, 0.1 g; autolyzed yeast, 5 cc of 1-percent extract; glucose, 60 g; redistilled water, 1,000 cc. The yeast and nutrient solution were transferred to fermentation tubes and incubated at 32° C. In this case the yeast was exposed to the dye at various hydrogen-ion concentrations, and since no fermentation took place during this exposure little change in reaction occurred. Fermentation by the treated yeast occurred at the same pH value, that of the nutrient solution, the pH of which was 5.9. By using this procedure the following results were secured.

EOSIN

Sodium phosphate buffer mixtures with the sodium constant at $\frac{N}{50}$ and ranging from pH 3.2 to pH 8.4 were prepared. Fifty cc quantities

of the buffer mixtures were placed, with sufficient eosin to make the dilution 1 to 5,000, in 150 cc Erlenmeyer flasks. Twenty-four drops of the standard yeast suspension were added to each flask. After 24 hours at room temperature the yeast had settled to the bottom of the flasks. The supernatant liquid was decanted and the color of the yeast examined. At pH 3.2, 3.6, and 3.9 the yeast was quite red; at pH 4.5, 5.0, and 5.5 lighter red; at pH 6.0, 6.5, 6.9, 7.5, 7.9, and 8.4, faint red with a slight gradation of color; and at pH 8.4 it was lightest in color. Forty cubic centimeters of the nutrient solution containing glucose was then added to each flask and the flask was shaken until all the yeast was in suspension. The contents of each flask were divided between two fermentation tubes, thus making duplicates for each reaction in the series. The tubes were incubated at 32° C. and gas production was measured at intervals. The results at the end of 4½ and 7 hours are shown in figure 2. The toxicity of eosin is greater in the acid solutions, the effect becoming noticeable in solutions more acid than pH 5.0. After 7 hours the fermentation tubes containing yeast exposed to eosin at hydrogen-ion concentrations of from pH 5.0 to pH 7.9 were entirely filled with gas while those containing yeast which had been exposed to eosin at pH 3.2 and 3.5 showed almost no gas production. As was noted with the acid dye, rose bengale, the toxicity of eosin increases with increasing hydrogen-ion concentration. This greater toxicity is correlated with the amount of dye absorbed by the yeast, the greater amounts of dye being absorbed in the more acid solutions.

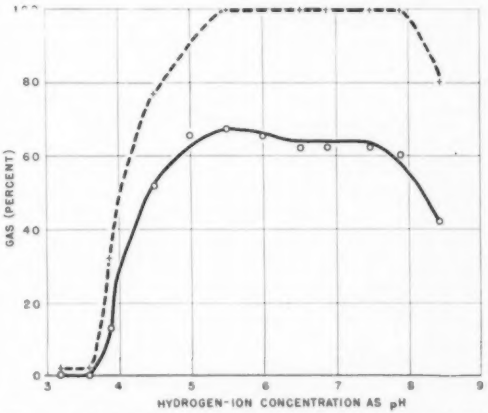

FIGURE 2.—Percentage of gas produced by yeast in 4½ hours (solid line) or in 7 hours (dotted line) at 32° C. in a nutrient solution containing 6-percent glucose. The yeast had been exposed for 24 hours in sodium phosphate buffer mixtures of various hydrogen-ion concentrations to eosin, 1 to 5,000. Percentage of gas plotted against pH at which yeast was exposed to eosin.

SAFRANINE

Using the same procedure as before, the yeast was exposed to the basic dye safranine, at a dilution of 1 to 25,000. When the buffer mixtures were decanted the yeast was colored as follows: At pH 3.1 and 3.6, light red; at pH 4.3, 5.1, and 6.0, gradually deeper red; at pH 6.5, 7.1, 8.0, and 8.8, deep red. The production of gas in the nutrient solution after 8 and 20 hours' incubation is shown in figure 3. The basic dye safranine is more injurious with decreasing hydrogen-ion concentration, the injurious effect under the conditions of the experiment becoming noticeable at reactions more alkaline than pH 6.0.

Observations of the fermentation tubes at the end of 20 hour showed that at pH 3.1, 3.6, 4.3, 5.1, and 6.0 the yeast was grow

and the contents of the tubes were cloudy as a result; little or no growth was evident at pH 6.5, 7.1, 8.0, and 8.8. Dilutions of safranine, 1 to 50,000, 1 to 100,000, and 1 to 500,000, also were used. Safranine at these dilutions for the period of exposure employed was not particularly toxic to yeast, and no differences in gas production by yeast exposed to the dye at various hydrogen-ion concentrations were noted.

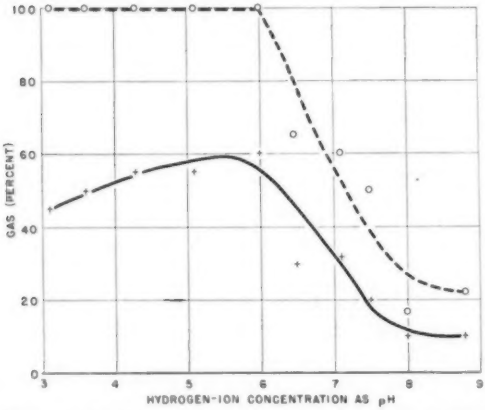

FIGURE 3.—Percentage of gas produced by yeast in 8 hours (solid line) or in 20 hours (dotted line) at 32° C. in a nutrient solution containing 6-percent glucose. The yeast had been exposed for 24 hours in sodium phosphate buffer mixtures of various hydrogen-ion concentrations to safranine, 1 to 25,000. Percentage of gas plotted against pH at which yeast was exposed to safranine.

DAHLIA

The basic dye dahlia was used at a dilution of 1 to 50,000. When the buffer mixtures were decanted, the yeast was deeply stained at all reactions. The gas production in the nutrient solution was observed after 8 and 20 hours; the results after 20 hours' incubation are shown in figure 4. The effect of hydrogen-ion concentration on the toxicity of dahlia to yeast is slight, much less than on that of safranine, eosin, or rose bengale. Dahlia is somewhat less toxic at pH 3.2 and 3.6 than at more alkaline reactions. Similar results were secured with dahlia at a dilution of 1 to 100,000.

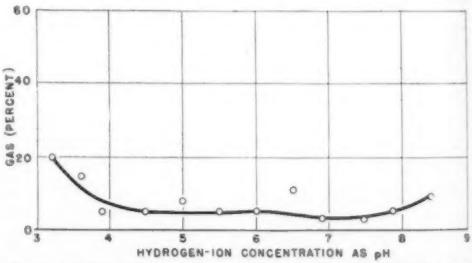

FIGURE 4.—Percentage of gas produced by yeast in 20 hours at 32° C. in a nutrient solution containing 6 percent glucose. The yeast had been exposed for 24 hours in sodium phosphate buffer mixtures of various hydrogen-ion concentrations to dahlia, 1 to 50,000. Percentage of gas plotted against pH at which yeast was exposed to dahlia.

BRILLIANT GREEN

The basic dye brilliant green was used at dilutions of 1 to 10,000 and 1 to 5,000. When the dye and buffer mixtures were decanted from the yeast exposed to brilliant green at a dilution of 1 to 10,000, the yeast was stained as follows: At pH 3.1, light green; at pH 3.6 and 4.3, deeper green; at pH 5.1, deepest green; at pH 6.3, lighter green, about the same color as yeast from pH 4.3; at pH 6.5, lighter green; at pH 7.1, 7.5, 8.0, and 8.8, successively lighter in color. The production of gas in the nutrient solution after 5 and 9½ hours by the yeast exposed to brilliant green, 1 to 10,000, is shown in figure 5. The absorption of the dye by the yeast at the various hydrogen-ion concentrations and the production of gas by the treated yeast, which

is correlated with the amount of dye absorbed, do not agree with the results anticipated for the influence of hydrogen-ion concentration on the effects of a basic dye. Dye absorption and injury increased as the acidity decreased from pH 3.1 to pH 5.1. Further decrease in acidity resulted in a decrease of dye absorption and of toxicity. The resulting curves for gas production show 2 maxima with a minimum at pH 5.1 between the 2 maxima.

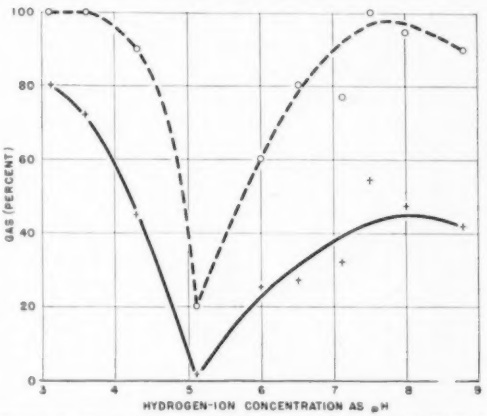

FIGURE 5.—Percentage of gas produced by yeast in 5 hours (solid line) or in 9½ hours (dotted line) at 32° C. in a nutrient solution containing 6 percent glucose. The yeast had been exposed for 24 hours in sodium phosphate buffer mixtures of various hydrogen-ion concentrations to brilliant green, 1 to 10,000. Percentage of gas plotted against pH at which yeast was exposed to brilliant green.

The gas production, after 5 and 9½ hours, by yeast exposed for 24 hours to brilliant green, 1 to 5,000, at various hydrogen-ion concentrations is shown in figure 6. The results are similar to those secured with brilliant green, 1 to 10,000. Marked injury is evidenced at somewhat more acid reactions than with the 1 to 10,000 dilution and the reduction in toxicity in the more alkaline reactions of the series is less marked.

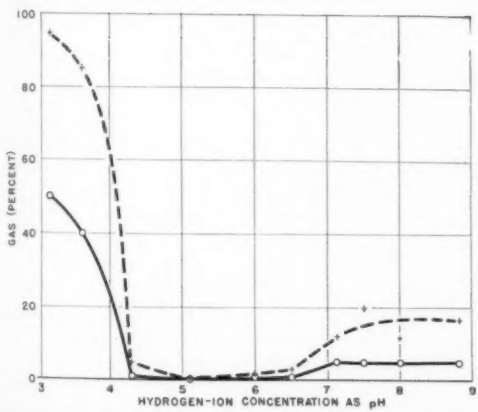

FIGURE 6.—Percentage of gas produced by yeast in 5 hours (solid line) or in 9½ hours (dotted line) at 32° C. in a nutrient solution containing 6 percent glucose. The yeast had been exposed for 24 hours in sodium phosphate buffer mixtures of various hydrogen-ion concentrations to brilliant green, 1 to 5,000. Percentage of gas plotted against pH at which yeast was exposed to brilliant green.

The behavior of brilliant green is due in part to the precipitation of the dye in the more alkaline solutions. When sufficient dye to make a dilution of 1 to 5,000 was added to buffer mixtures in test tubes a precipitate was observed at pH 5.5, 6.0, 6.5, 6.9, 7.5, 7.9, and 8.4.

The effect of hydrogen-ion concentration upon the toxicity of brilliant green to yeast is the result of the influence of reaction on the toxicity of the basic dye, which is increased by decreasing hydrogen-ion concentration, and the result of the influence of reaction on the solubility of the dye, which is decreased by decreasing hydrogen-ion

concentration. As the hydrogen-ion concentration is decreased from pH 3.1, the basic dye, brilliant green, becomes more toxic. In the vicinity of pH 5.5, however, the dye is precipitated. As a result further decrease in hydrogen-ion concentration decreases the toxicity of the dye. This probably explains the results reported by Stearn and Stearn (*16, pp. 349–350*) on the influence of hydrogen-ion concentration upon the toxicity of brilliant green to bacteria.

DISCUSSION

The experiments reported in this paper show that hydrogen-ion concentration is a factor which cannot be ignored in a study of the toxicity of compounds to living organisms. When hydrogen-ion concentration does not affect the solubility of the dye within the range of reactions studied, increasing hydrogen-ion concentration increases the toxicity of acid dyes, and decreasing hydrogen-ion concentration increases that of basic dyes. Since for the acid dyes the toxic ion is the anion and for the basic dye it is the cation, the influence of hydrogen-ion concentration upon the toxicity of acid and basic dyes agrees with the generality stated at the beginning of this paper, namely, that the toxicity of anions is increased by increasing hydrogen-ion concentration and that of cations is increased by decreasing hydrogen-ion concentration. The degree to which hydrogen-ion concentration affects toxicity varies with the dye, as is shown by the differences found between dahlia and the other dyes used.

The effect of hydrogen-ion concentration upon toxicity has been variously ascribed to its influence upon the organism by changing its permeability (*1*) or the combining capacity of its constituents for anions and cations (*11, 13, 14, 15*), or some other property of the cell (*10, 7*); to its influence upon the toxic substance by affecting its surface tension (*17, 18*), dissociation (*1*), solubility (*9*), partition coefficient (*9*), or formation of free acid or free base (*2, 8*); to a combination of effects upon the cell and upon the toxic substance (*1*). Until further evidence is at hand the simplest and most reasonable explanation for the effect of hydrogen-ion concentration upon the toxicity of acid and basic dyes is to assume that the most toxic form of the dye is the free base or free acid and that hydrogen-ion concentration affects the toxicity through its influence upon the production of free base and free acid.

SUMMARY

The influence of hydrogen-ion concentrations, ranging from about pH 3.0 to about pH 8.0, on the effect of rose bengale, eosin, safranine, dahlia, and brilliant green on yeast fermentation was studied.

Under the conditions of the experiments the toxicity of rose bengale and of eosin was greater in the more acid solutions, the effect becoming noticeable in solutions more acid than pH 6.0 and pH 5.0, respectively; the toxicity of safranine was greater in the less acid solutions, the injurious effect becoming noticeable at reactions more alkaline than pH 6.0.

The toxicity of dahlia was least in the most acid solutions used. However, the influence of hydrogen-ion concentration on the toxicity of this dye was not marked.

The toxicity of brilliant green under the conditions of the experiments increased with decrease in hydrogen-ion concentration up to about pH 5.5, where precipitation of the dye occurred.

In general increasing hydrogen-ion concentration increased the toxicity of the acid dyes, and decreasing hydrogen-ion concentration increased that of the basic dyes.

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THE RELATION OF SEXUAL MATURITY AND EGG WEIGHT IN THE DOMESTIC FOWL[1]

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INTRODUCTION

Early sexual maturity and the production of large eggs are both desirable characteristics in the domestic fowl. The breeder is interested in establishing these qualities in his stock, and any information which contributes to a knowledge of them should be beneficial to him. This paper reports a study of the relationships which exist between age at sexual maturity and date of sexual maturity and (1) mean weight of the first 10 eggs laid, (2) maximum mean monthly egg weight, and (3) mean annual egg weight. The interrelationships of these egg weights were measured by correlation coefficients.

SOURCE OF DATA

The data used for this investigation were obtained from records on pullets which were hatched in 10 consecutive weekly hatches beginning the first week in February in 1931 and 1932. Each hatch was brooded separately, but all were fed the same rations and received the same general care. High summer temperatures and a lack of green feed during July and August were more depressing to the growth of the chicks hatched later than to those hatched earlier in the season. Whether the growth rate and the size of the egg subsequently laid by a pullet are associated has not been established.

Only those pullets that were considered in good health and suitable for laying purposes, were housed but selection was not rigid. The White Leghorns and Rhode Island Reds were fed similar rations, housed in the same type of houses, and given the same general care and management. The White Plymouth Rocks were fed experimental rations, but there was no significant differences in the size of eggs produced by the different pens. The egg weights for the White Leghorns and Rhode Island Reds were determined by weighing the eggs laid on 4 consecutive days of each month for a period of 10 months. All eggs laid by the White Plymouth Rock pullets from the first egg laid in the fall of 1932 to June 30, 1933, were weighed. All eggs were weighed to the nearest gram. Mean egg weight for these periods was designated mean annual egg weight.

There were 122 complete records available for the study of egg weight in the White Plymouth Rock pullets. Sufficient data were available for the correlation of age in days at sexual maturity and mean annual egg weight for 104 White Leghorn pullets and 129 Rhode Island Red pullets. A total of 154 records on the Rhode Island Reds contained sufficient data for determining egg weight by months.

[1] Received for publication May 14, 1934; issued February 1935. Contribution from the Department of Poultry Husbandry, Missouri Agricultural Experiment Station. Journal Series No. 382.

PRESENTATION AND DISCUSSION OF DATA

The relation of age at sexual maturity and egg weight has been statistically analyzed by a number of investigators. In the cases reported no attempts were made to eliminate the influence of factors associated with age at sexual maturity and thus arrive at the influence of age alone. If these associations are fairly constant, possibly a more detailed analysis may not be necessary but merely desirable in order to arrive at the basic relationships. Those birds which mature at an early age are usually the smaller ones. They also attain maturity at an earlier date than do those which are older when they reach sexual maturity. Therefore before concluding that age at sexual maturity and egg weight are related it would be advisable to eliminate the influence of (1) body weight at sexual maturity and (2) date of sexual maturity. An attempt has been made in this study to eliminate the influence of these two factors and thus to measure more accurately the relationship of age at sexual maturity and egg weight.

The simple correlation coefficient for age at sexual maturity and mean weight of the first 10 eggs laid by White Plymouth Rock pullets was $r_{12}=0.571$. For this same relationship Lippincott, working with Leghorns (*10*),[2] found $r=0.72\pm0.03$. Atwood (*1*) reported for Leghorns six coefficients ranging from $r=-0.07\pm0.15$ to $r=0.68\pm0.05$. Jull (*6*) reported for Barred Rocks $r=0.845\pm0.03$. Parkhurst (*14*) found for White Leghorns $r=0.723\pm0.039$, and Godfrey (*3*) reported for White Leghorns $r=0.68$. Upp and Thompson (*16*) correlated age at sexual maturity and weight of the first dozen eggs laid and found $r=0.789\pm0.015$. Callenbach (*2*) has shown that there is a moderate but significant positive correlation between age at sexual maturity and weight of the first egg. Hays (*5*) found in Rhode Island Reds that age at first egg and mean egg weight to January 1 were related by $r=0.421\pm0.015$. Lippincott, Parker, and Schaumburg (*11*) showed that age at sexual maturity and mean egg weight for the first twelve 30-day periods were positively correlated. It would thus appear that in chickens, age at sexual maturity and the mean weight of the first 10 eggs laid are quite closely related.

Since birds that are older at sexual maturity tend to be larger (this relationship is shown by $r_{16}=0.615$) and also to mature at a later date (this relationship is shown by $r_{15}=0.730$), the apparent influence of age at sexual maturity and mean weight of the first 10 eggs laid may be due partly to the influence of these two associated factors. By means of partial correlation an attempt was made to eliminate the influence of these two factors. The results with White Plymouth Rock pullets are shown in the following tabulation:

Correlation of mean weight of the first 10 eggs laid (X_2) and—	*Coefficient*[1]
X_1 Age in days at sexual maturity	$r_{12}=0.571$
Eliminating the influence of body weight at sexual maturity	$r_{12.6}=0.340$
Eliminating the influence of date of sexual maturity	$r_{12.5}=0.388$
Eliminating the influence of body weight and date of sexual maturity	$r_{12.56}=0.275$

[1] Reference is made by number (italic) to Literature Cited, p. 1038.

[2] Significance of the values of r may be determined by comparing them with the following values listed by Wallace and Snedecor (*17*):

Size of sample:	Chance occurrence of r in an uncorrelated population	
	5 in 100 cases	1 in 100 cases
127	$r=0.174$	$r=0.228$

	Coefficient
Correlation of mean weight of the first 10 eggs laid (X_2) and—Con.	
X_5 Date of sexual maturity	$r_{25}=0.453$
Eliminating the influence of body weight at sexual maturity	$r_{25.6}=0.208$
Eliminating the influence of age at sexual maturity	$r_{25.1}=0.076$
Eliminating the influence of body weight and age at sexual maturity	$r_{25.16}=0.009$
Correlation of mean annual egg weight (X_3) and—	
X_1 Age in days at sexual maturity	$r_{13}=0.235$
Eliminating the influence of body weight at sexual maturity	$r_{13.6}=0.023$
Eliminating the influence of date of sexual maturity	$r_{13.5}=0.226$
Eliminating the influence of body weight at sexual maturity and date of sexual maturity	$r_{13.56}=0.110$
X_5 Date of sexual maturity	$r_{35}=0.103$
Correlation of maximum mean monthly egg weight (X_4) and—	
X_1 Age at sexual maturity	$r_{14}=0.103$
X_5 Date of sexual maturity	$r_{45}=0.010$
Correlation of age at sexual maturity (X_1) and—	
X_5 Date at sexual maturity	$r_{15}=0.730$
X_6 Body weight at first egg	$r_{16}=0.615$

Eliminating the influence of body weight at sexual maturity gave $r_{12.6}=0.340$, eliminating the influence of date of sexual maturity gave $r_{12.5}=0.388$, and eliminating the influence of both of these factors gave $r_{12.56}=0.275$. From these results it was evident that the age at which the pullet began to lay was a factor influencing the weight of the first 10 eggs laid even though the influence of body weight and date of sexual maturity were removed. There may be other factors associated with age at sexual maturity which should be eliminated before concluding that the age of the bird is associated with the weight of the first 10 eggs laid. Until these influences have been detected and removed one may conclude that age of the pullet influences the weight of the first 10 eggs laid.

Maximum mean monthly egg weight attained during the first year's production by White Plymouth Rocks was not significantly correlated with age at sexual maturity ($r_{14}=0.103$) or with date of sexual maturity ($r_{45}=0.010$). Evidently neither the age at which these pullets began to lay nor the date of their sexual maturity had any influence on the maximum egg size attained.

The age at which the pullets became sexually mature and mean annual egg weight were correlated as follows: White Leghorns $r_{13}=0.13$,[4] Rhode Island Reds $r_{13}=0.08$, and White Plymouth Rocks $r_{13}=0.235$. The correlation in the White Plymouth Rocks was significant, but as will later be shown, it was not a causal relationship. Atwood (*1*) reported for this relationship in White Leghorns four correlation coefficients ranging from $r=-0.03\pm0.15$ to $r=0.33\pm0.12$, and Marble (*12*) reported for this relationship in the same breed $r=0.017\pm0.024$, $r=0.280\pm0.037$, and $r=0.067\pm0.036$. Table 1 shows that by analyzing the data for the White Plymouth Rocks by a frequency distribution there does appear to be some association between age at sexual maturity and mean annual egg weight. This frequency distribution does not eliminate, however, the influence of body weight. Graham, cited by Jull (*8*), has shown by a frequency distribution that in the Rhode Island Reds kept at the Massachusetts station early sexual maturity and small egg size were related. In

[4] Correlation coefficients for White Leghorns were based on 104 records; those for Rhode Island Reds on 129 records.

the present study eliminating the influence of body weight at sexual maturity gave $r_{13.6}=0.023$, while removing the influence of date of sexual maturity gave $r_{13.5}=0.226$, and eliminating the influence of both body weight and date at sexual maturity gave $r_{13.56}=0.110$. It seems logical to conclude from these results that the age at which the pullets began to lay was only indirectly related to mean annual egg weight.

TABLE 1.—*Relation of age at first egg and egg weights for White Plymouth Rock pullets, 1932–33*

Age at first egg (days)	Birds	Mean weight first 10 eggs	Mean annual egg weight	Maximum monthly egg weight
	Number	*Grams*	*Grams*	*Grams*
151 to 200	23	49.1	54.9	57.5
201 to 225	32	50.8	54.9	57.4
226 to 250	41	55.0	56.8	58.7
251 to 325	26	55.9	56.8	58.4

The date of sexual maturity in Rhode Island Reds was definitely associated with the weight of the eggs laid during the first few months' production, but apparently was not related to the mean egg weight of the first 10 months' production. Table 2 shows that maximum mean monthly egg size was reached in February by Rhode Island Red pullets which began laying in any month from September to January. Birds which began production in September required 5 months to produce eggs of maximum size, while birds which reached sexual maturity in January were producing eggs of maximum size in February. Hays (*5*) found that in Rhode Island Reds maximum egg size was reached in February. Jull (*7*) has also shown this phenomenon to exist in Barred Plymouth Rock pullets. Evidently there was a tendency for pullets to lay eggs of maximum size during the natural breeding season. Parkhurst (*15*) presented data from England which showed that maximum egg size was not reached until May–June by pullets which began laying in August–September. Birds which become sexually mature during the early fall months are in many cases smaller and younger than those which begin laying later. Thus the specific influence of date of maturity would be obscured by the influence of these two factors. It will be observed in table 2 that the date of sexual maturity was not related to the mean egg weight of the first 10 months' production of the Rhode Island Red pullets.

The date at which the White Plymouth Rock pullets began to lay was significantly related to the weight of the first 10 eggs laid ($r_{25}=0.453$). However, removing the influence of body weight at sexual maturity gave $r_{25.6}=0.208$, removing the influence of age at sexual maturity gave $r_{25.1}=0.076$, and removing the influence of both body weight and age at sexual maturity gave $r_{25.16}=0.009$, thus completely eliminating the influence which appeared to be due to the date of sexual maturity. Table 2 shows that in Rhode Island Reds there appeared to be an association between the date the birds began to lay and the size of the eggs laid during the first few months' production. In view of the foregoing correlation coefficients it would appear

that the apparent influence of date of sexual maturity on egg weight was in reality the influence of body weight and age at sexual maturity.

TABLE 2.—*Relation of date of sexual maturity and the weight of eggs produced by Rhode Island Red pullets*

Month of sexual maturity	Birds	Average individual egg weight by month indicated—										Mean of the period
		First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth	Tenth	
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
September	14	48.4	51.3	55.4	58.0	57.4	61.2	61.0	60.4	58.4	59.2	57.1
October	19	50.9	54.5	56.6	57.3	59.6	58.4	57.7	56.7	56.9	56.3	56.5
November	50	54.6	56.4	57.0	58.4	58.2	57.1	57.8	56.5	53.5	57.5	56.7
December	39	56.3	56.0	58.2	57.2	55.3	55.1	55.0	53.0	55.6	57.8	56.0
January	32	57.8	59.2	57.8	56.3	55.4	55.6	52.8	57.0	58.3	56.7	56.7

Definite interrelationships exist between egg size during different periods of the first year's production, as is shown in the following tabulation of data on White Plymouth Rock pullets:

Correlation of mean weight of the first 10 eggs laid (X_2) and the mean annual egg weight (X_3) ..... $r_{23}=0.828$

Correlation of mean weight of the first 10 eggs laid (X_2) and the maximum monthly egg weight (X_4) ..... $r_{24}=0.7180$

Correlation of maximum monthly egg weight (X_4) and mean annual egg weight (X_3) ..... $r_{34}=0.949$

The mean weight of the first 10 eggs laid was closely related to mean annual egg weight and also to maximum mean monthly egg weight. The correlation of mean annual egg weight and maximum mean monthly egg weight was $r_{34}=0.949$. Hadley and Caldwell (*4*) found that the size of the first egg laid was associated in a positive manner with mean annual egg weight and also with what he termed "maximum" eggs. Jull and Godfrey (*9*) have shown that White Leghorns which lay large eggs when they begin production have a higher mean annual egg weight than do birds which lay small eggs when they begin production. Wilson and Warren (*18*) have shown that there is a very definite relation between the mean weight of the first eggs produced and maximum egg weight. Maw and Maw (*13*) reported that a pullet must have an average weight of 47.5 g for the first 10 eggs in order to lay eggs of standard size before the end of the pullet year.

SUMMARY AND CONCLUSIONS

There was a significant positive correlation ($r_{12}=0.571$) between age at sexual maturity and the mean weight of the first 10 eggs laid by White Plymouth Rock pullets. By eliminating the influence of body weight at sexual maturity and date of sexual maturity the size of the coefficient of correlation was reduced, but there remained a significant positive relationship between these two factors (X_1 and X_2). Apparently young birds did not produce as large eggs when they began production as did older birds even though there was no difference in body weight.

Age at sexual maturity and maximum mean monthly egg weight were not significantly related in the White Plymouth Rock pullets ($r_{14}=0.103$).

Age at sexual maturity and mean annual egg weight were not significantly related in White Leghorns ($r_{13}=0.13$) or in Rhode Island Reds ($r_{13}=0.08$), but they were significantly related in White Plymouth Rocks ($r_{13}=0.235$). Eliminating the influence of body weight at sexual maturity from this relationship gave $r_{13.6}=0.023$, thus indicating that age at sexual maturity was related to mean annual egg weight only indirectly and because it was correlated with body weight ($r_{16}=0.615$).

Date of sexual maturity, while definitely associated with the weight of the first eggs produced ($r_{25}=0.453$) by the White Plymouth Rock pullets, did not exert any specific effect on egg size, for when the influence of age at sexual maturity was removed from this relationship the coefficient of correlation became $r_{25.1}=0.076$.

There was no relation between date of sexual maturity and mean annual egg weight in the White Plymouth Rocks or Rhode Island Reds.

Mean weight of the first 10 eggs laid and mean annual egg weight were closely related ($r_{23}=0.828$) in the White Plymouth Rock pullets.

Mean weight of the first 10 eggs and maximum mean monthly egg weight were related by $r_{24}=0.718$.

Mean annual egg weight and maximum mean monthly egg weight were quite closely related ($r_{34}=0.949$).

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THE EFFECT OF FLUORINE FEEDING ON THE STORAGE OF VITAMIN C IN THE RAT AND GUINEA PIG[1]

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INTRODUCTION

It has been observed *(3)*[3] that hemorrhages of the pyloric mucosa are relatively common in rats suffering from chronic fluorine poisoning produced by feeding sodium fluoride at a level of 0.15 percent. Moreover, it is now recognized *(12)* that mottled enamel results from the ingestion of fluorides, and that this disturbance is characterized by the lack of intercementing material normally found between the enamel rods *(1)*. These observations suggested a similarity between fluorine poisoning and certain features of scurvy.

At the University of Wisconsin, in 1931–32[4] an attempt was made to determine whether a vitamin C supplement to the ration would mitigate the effects of fluorine feeding. Five rats weighing from 54 to 70 g were given 5 cc of orange juice each, per day, in addition to a stock ration to which 0.15 percent sodium fluoride had been added. Specifically, the stock ration was composed of parts by weight as follows: Yellow corn 71.5, linseed meal 15.0, crude casein 5.0, alfalfa 2.0, sodium chloride 0.5, calcium phosphate 1.0, butterfat 21.0, skim-milk powder 34.0, yeast 3.0, and cod-liver oil 3.0. Five control rats were given the same ration with sodium fluoride, but the orange-juice supplement was omitted. The experiment was continued for 29 weeks, at which time the average weight of four surviving rats which had received orange juice and fluoride was 215 g. Only one control rat, weighing 177 g, survived until the termination of the experiment. Apparently some factor in the orange-juice supplement influenced the growth and survival of rats subjected to chronic fluorine poisoning. However, despite the better growth and longer survival period of the rats receiving orange juice, the symptoms commonly observed in rats with chronic fluorine poisoning, such as the tooth changes, hemorrhages in the pyloric mucosa, and kidney degeneration, were evident. Possibly at a lower level of fluorine intake the appearance of these symptoms might have been delayed or suppressed by the administration of orange juice.

Phillips *(9)* was led to investigate the influence of sodium fluoride upon guinea pigs fed a scorbutic diet fortified with graded levels of orange juice. Several times the normal antiscorbutic dose of orange juice did not suffice to prevent the appearance of scurvylike symptoms in guinea pigs fed 25 to 30 mg of fluorine per kilogram of body weight. This author made the tentative suggestion that fluorine poisoning might be an interference with the action of vitamin C in the organism.

[1] Received for publication June 21, 1934; issued February 1935.
[2] The author wishes to acknowledge the courtesy of Prof. L. A. Maynard, of the laboratory of animal nutrition of the Department of Animal Husbandry, Cornell University, in providing laboratory facilities, and the assistance of J. C. Woodward in carrying out these experiments.
[3] Reference is made by number (italic) to Literature Cited, p. 1046.
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A study was undertaken to determine whether fluorine feeding interferes with storage of vitamin C in rats and guinea pigs. These animals were chosen because it has been demonstrated that the livers of rats, even when the rats have been fed a ration which would produce scurvy in guinea pigs, contain appreciable amounts of vitamin C (*4*, *7*) and also that the livers of guinea pigs on a ration adequate in vitamin C are antiscorbutic (*8*).

EXPERIMENTAL STUDY OF VITAMIN C STORAGE IN FLUORINE-FED RATS

Rats from 3 to 5 months of age were fed a stock ration to which 0.15 percent sodium fluoride had been added. A second group of rats received the stock ration only. Specifically, the stock ration (*5*) consisted of linseed meal 15 parts by weight, ground malted barley 10, wheat red dog flour 22, dried skim milk 15, oat flour 15, yellow corn meal 20, steamed bone meal 1, ground limestone 1, sodium chloride 1, and cod-liver oil 2. In the group of rats receiving sodium fluoride typical changes in the incisors were evident in from 2 to 4 weeks. The fluorine feeding was continued from 10 to 13 weeks before the rats were killed with chloroform to furnish supplements for scorbutic guinea pigs. Hemorrhages of the pyloric mucosae, which occur commonly in rats suffering with chronic fluorine poisoning, were observed in these animals.

In order to assay the livers and adrenals of these rats for vitamin C, young guinea pigs, which weighed from 250 to 300 g, were prepared by a preliminary period on a scorbutic ration (*4*) supplemented by cabbage. The scorbutic ration consisted of rolled oats 69 parts by weight, autoclaved alfalfa 25 (autoclaved 30 minutes at 15 pounds pressure), casein 5, and sodium chloride 1. After 8 days the cabbage was discontinued, and the scorbutic ration alone fed for from 7 to 11 days, until a decline in weight occurred. Supplements were then fed, as indicated in table 1.

TABLE 1.—*Summary of autopsy findings in guinea pigs fed rat tissues in addition to basal scorbutic ration*

Supplement	Guinea pig no.	Hemorrhages at joints	Enlargement of costochondral junctions	Teeth loose
None	16	Marked [1]	+	+
	22	Do.	+	+
	23	Do.	+	+
3 cc orange juice	1	—	—	—
	12	—	—	—
	15	—	—	—
3 g liver from rat fed on stock ration only	10	—	—	—
	17	—	—	—
3 g liver from fluorine-fed rat	3	—	—	—
	8	—	—	—
1 g liver from rat fed on stock ration only	7	—[1]	—	—
	11	Marked	+	—
1 g liver from fluorine-fed rat	2	Do.	+	—
	9	Do.	+	—
Adrenals from rat fed on stock ration only	20	Moderate	+	[2]
Adrenals from fluorine-fed rat	18	Marked	+	—

[1] Hemorrhages in intestine
[2] Lower incisors only.

It was desired to use levels sufficiently low so that differences in vitamin C content between the livers of fluorine-fed and stock rats would be detectable. One- and three-gram levels of liver were chosen, since Lepkovsky and Nelson (*4*) had found that these amounts of liver from rats on a ration which would produce scurvy in guinea pigs sufficed for the recovery of guinea pigs from scurvy. Fresh rats' livers were used for each feeding. Portions were weighed, cut into pieces, and hand-fed to the guinea pigs.

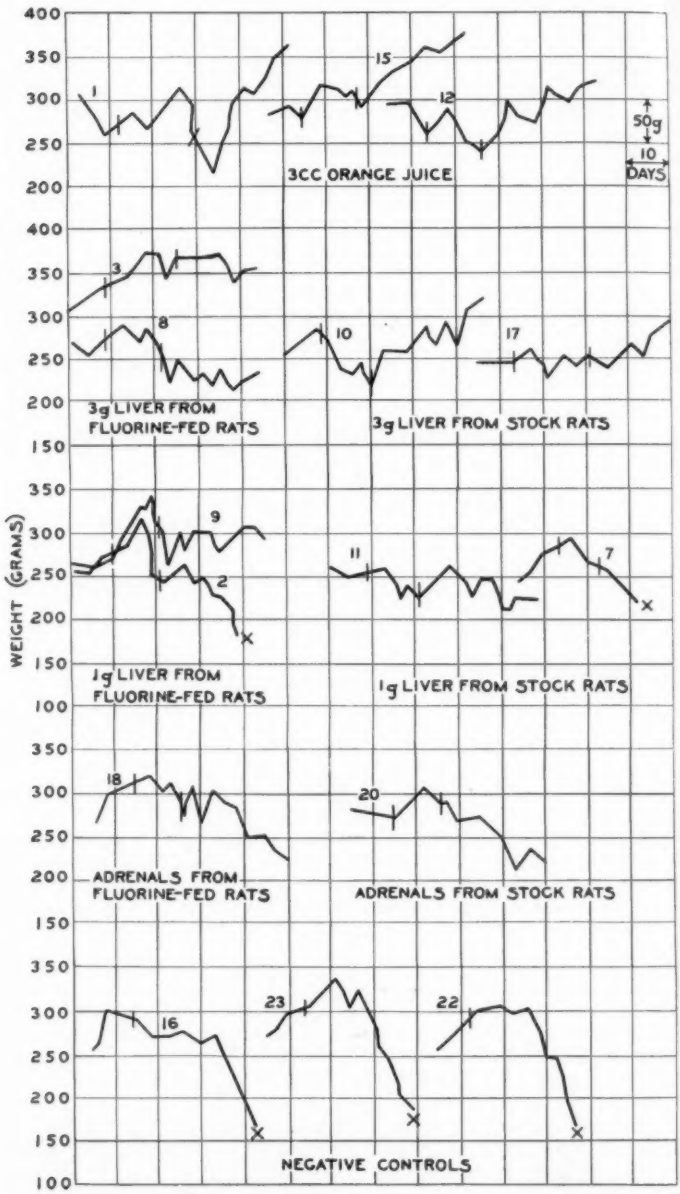
The supplementary feedings were continued for 26 days. The three negative controls died from 10 to 14 days before the conclusion of the experiment, and all showed marked evidence of scurvy. The surviving guinea pigs were killed at the termination of the experiment, and were examined carefully for gross evidences of scurvy. The silver nitrate staining test was applied to the cut adrenals. The only adrenals that reduced silver nitrate were those from the three positive controls which had received 3 cc of orange juice daily. This indicates that the liver-fed guinea pigs, although receiving enough vitamin C to protect them from scurvy, had not enough to permit storage of vitamin C in the adrenals. This is in harmony with the observation of Hamilton (*2*) that storage of vitamin C in the adrenal does not occur until there is a surplus over the metabolic needs.

Moore and Ray have previously reported (*6*) that the adrenals of rats on a ration which would be scorbutic for guinea pigs stain deeply with silver nitrate. Therefore small sections of the rat adrenals were tested with 0.4 percent silver nitrate solution according to the technic of Szent Györgyi (*13*). All of these sections had a strong reducing action. The remaining portions of adrenal, weighing from 0.03 to 0.06 g, were fed as a supplement to the scorbutic ration.

No difference was demonstrated between the antiscorbutic value of the livers or of the adrenals from fluorine-fed and from stock rats. The weight curves of the guinea pigs are shown in figure 1. As indicated in table 1, the autopsy findings were quite similar for comparable groups. Guinea pigs receiving 3 g of liver from either source showed no gross signs of scurvy. One gram of liver, whether from fluorine-fed or stock rats, afforded incomplete protection against scurvy. Although the amounts of adrenal tissue available were insufficient to protect fully against scurvy (1.02 g stock adrenals and 1.26 g adrenals from fluorine-fed rats for the 24-day period) its antiscorbutic activity was clearly demonstrated by the greater survival period and better condition at autopsy of guinea pigs receiving adrenals as compared with the negative controls.

EXPERIMENTAL STUDY OF VITAMIN C STORAGE IN FLUORINE-FED GUINEA PIGS

A similar experiment was conducted to determine whether vitamin C storage in the guinea pig is affected by fluoride feeding. Young guinea pigs, weighing 200 to 300 g, were fed approximately 25 mg of fluorine per kg plus 3 cc of orange juice daily in addition to the basal scorbutic ration described above. The fluorine was fed as a 4-percent solution of sodium fluoride, given by pipette immediately preceding the orange juice. A control group was fed the basal scorbutic ration with the orange-juice supplement only. Tissues from these animals were later fed as supplements to a group of scorbutic guinea pigs.


WEIGHT (GRAMS)
400
350
300
250
200
150
100
1
15
12
50g
10
DAYS
3CC ORANGE JUICE
3
8
10
17
3g LIVER FROM FLUORINE-FED RATS
3g LIVER FROM STOCK RATS
9
2
11
7
1g LIVER FROM FLUORINE-FED RATS
1g LIVER FROM STOCK RATS
18
20
ADRENALS FROM FLUORINE-FED RATS
ADRENALS FROM STOCK RATS
16
23
22
NEGATIVE CONTROLS

The animals had received the fluoride for from 22 to 34 days before they were killed. The only scurvylike symptom observed was poor control of the hind legs in one animal. Unfortunately, accidental chilling of the animals resulted in a number of deaths from lung infection, both among the controls and the fluorine-fed animals, so that the subsequent tests for vitamin C in the tissues were limited to 9 days.

TABLE 2.—*Summary of autopsy findings in guinea pigs fed guinea-pig tissues in addition to basal scorbutic ration*

Supplement	Guinea pig no.	Autopsy findings
None	58	Moderate hemorrhages over knees, and around costochondral junctions.
	63	Severe hemorrhages over ribs and sternum.
3 g liver from control guinea pig	62	No evidence of scurvy.
	65	Do.
	66	Do.
3 g liver from fluorine-fed guinea pig	53	Lungs congested. No evidence of scurvy.
	54	Do.
	55	No evidence of scurvy.
	67	Slight hemorrhage over right knee.
1 adrenal from control guinea pig	57	Moderate hemorrhages over knees.
½ adrenal from control guinea pig	64	Lungs congested. No evidence of scurvy.
1 adrenal from fluorine-fed guinea pig	56	Slight congestion of lungs. No evidence of scurvy.
½ adrenal from fluorine-fed guinea pig	61	No evidence of scurvy.

In order to assay the livers and adrenals of these animals for vitamin C, scorbutic guinea pigs were prepared as in the previous experiment. Table 2 shows the supplements fed to the several groups and the autopsy findings. Although no quantitative comparison can be made on the basis of these limited data, it is evident that 3 to 5 weeks' fluorine feeding did not deplete the vitamin C content of the livers and adrenals of these guinea pigs to any great extent, if at all.

SUMMARY

No difference was observed between the vitamin C content of livers and adrenals of fluorine-fed and control rats.[5]

Adrenals of rats which were tested for vitamin C by the silver nitrate staining test, reduced silver nitrate rapidly, whether the adrenals were from fluorine-fed or control rats.

Data are presented showing that 3 to 5 weeks' fluorine feeding did not prevent storage of vitamin C in the adrenals and livers of guinea pigs.[5]

If fluorine feeding results in an interference with the action of vitamin C in the organism, such interference probably occurs elsewhere in metabolism rather than in storage of the vitamin.

[5] Since this was written, Phillips and Chang (*10*) and Phillips, Stare, and Elvehjem (*11*) have presented evidence obtained by titration with 2—6 dichloro-phenolindophenol, leading to similar conclusions.

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